Therapeutic effect of anti-OX40L and anti-TNF-α MAbs in a murine model of chronic colitis

T. Totsuka,1 T. Kanai,1 K. Uraushihara,1 R. Iiyama,1 M. Yamazaki,1 H. Akiba,2 H. Yagita,2 K. Okumura,2 and M. Watanabe1

1Department of Gastroenterology and Hepatology, Graduate School of Medicine, Tokyo Medical and Dental University, Tokyo 113–8519; and 2Department of Immunology, Juntendo University School of Medicine, Tokyo 113–8421, Japan

Submitted 21 October 2002; accepted in final form 27 December 2002

Totsuka, T., T. Kanai, K. Uraushihara, R. Iiyama, M. Yamazaki, H. Akiba, H. Yagita, K. Okumura, and M. Watanabe. Therapeutic effect of anti-OX40L and anti-TNF-α MAbs in a murine model of chronic colitis. Am J Physiol Gastrointest Liver Physiol 284: G595–G603, 2003. —Interaction of OX40 (CD134) on T cells with its ligand (OX40L) on antigen-presenting cells has been implicated in pathogenic T cell activation. This study was performed to explore the involvement of OX40/OX40L in the development of T cell-mediated chronic colitis. We evaluated both the preventive and therapeutic effects of neutralizing anti-OX40L MAb on the development of chronic colitis in SCID mice induced by adoptive transfer of CD4+CD45RBhigh T cells as an animal model of Crohn’s disease. We also assessed the combination of anti-OX40L and anti-TNF-α MAbs to improve the therapeutic effect. Administration of anti-OX40L MAb markedly ameliorated the clinical and histopathological disease in preventive and therapeutic protocols. In vivo treatment with anti-OX40L MAb decreased CD4+ T cell infiltration in the colon and suppressed IFN-γ, IL-2, and TNF-α production by lamina propria CD4+ T cells. The combination with anti-TNF-α MAb further improved the therapeutic effect by abolishing IFN-γ, IL-2, and TNF-α production by lamina propria CD4+ T cells. Our present results suggested a pivotal role of OX40/OX40L in the pathogenesis of T cell-mediated chronic colitis. The OX40L blockade, especially in combination with the TNF-α blockade, may be a promising strategy for therapeutic intervention of Crohn’s disease.

OX40L; tumor necrosis factor-α; Crohn’s disease; therapy

CROHN’S DISEASE (CD) is a chronic inflammatory bowel disease characterized by massive infiltration of CD4+ T cells and macrophages in the colon. Although its etiology remains unclear, it has been established that proinflammatory cytokine production by infiltrating T cells and macrophages plays a pivotal role in the pathogenesis of CD (9, 36). Functional activation of T cells requires two distinct signals: one derived from T cell receptor (TCR)-mediated recognition of peptide-major histocompatibility complex on antigen-presenting cells (APC) and another, designated as the costimulatory signal, derived from the interaction of a costimulatory molecule (such as CD28) on T cells and its ligand (such as B7) on APC (4, 6, 16, 30, 39). Although the CD28/B7 interaction constitutes a predominant pathway of T cell costimulation, some intact T cell responses in CD28-deficient mice have suggested the presence of alternative pathways (39).

Some members of the TNF receptor (TNFR) superfamily have been implicated in T cell costimulation, including CD27, 4–1BB (CD137), and OX40 (CD134) (7, 12, 14, 29, 40). OX40 is primarily expressed on T cells on TCR-mediated stimulation (2, 11, 24). Its ligand (OX40L) is a type II membrane protein belonging to the TNF family and is expressed on activated B cells, dendritic cells, and endothelial cells (2, 10, 34, 42). A number of in vitro studies have shown that OX40/OX40L interaction provides a costimulatory signal resulting in enhanced T cell proliferation and cytokine production (2, 3). It has also been shown that OX40+ T cells preferentially accumulated in inflammatory sites associated with various diseases and disease models, including rheumatoid arthritis (3), inflammatory skin diseases (26), graft vs. host disease (45), experimental autoimmune encephalomyelitis (EAE) (47), and murine inflammatory bowel disease models (13), suggesting pathogenic roles of the OX40/OX40L interaction. Recently, Malmstrom et al. (25) reported that intestinal inflammation in CD4+CD45RBhigh T cell-transferred colitic mice was characterized by a marked increase in the number of OX40L+ dendritic cells in the mesenteric lymph nodes (MLNs). Their finding that anti-OX40L treatment led to retarded T cell proliferation and expression of activation antigens in the MLN suggested that OX40L expression on dendritic cells plays an important role in driving the T cell response in CD4+CD45RBhigh T cell-transferred colitic model. Accumulating evidences have suggested that TNF-α plays a pivotal role in the development of mucosal inflammation in CD. First, disregulated expression of TNF gene in mice led to the development of inflammatory bowel disease reminiscent of human CD (19). Second, in several animal models of CD, administr-
tion of neutralizing anti-TNF-α MAb or TNF-α deficiency significantly ameliorated the mucosal inflammation (31). More importantly, dramatic improvement was achieved in approximately two-thirds of the patients with CD by a single infusion of anti-TNF-α MAb (Infliximab) (44, 46). Although these results support the pivotal role of TNF-α, the refractoriness to Infliximab observed in one-third of the CD patients suggests an alternative proinflammatory mechanism that may be mediated by the other members of TNF/TNFR family including the OX40/OX40L.

In this study, we used a chronic colitis model, which was induced by adoptive transfer of CD4+CD45RBhigh T cells to SCID mice (37), to characterize the ameliorating effects of anti-OX40L MAb in both preventive and therapeutic settings. In addition, we also assessed the combination of anti-OX40L and anti-TNF-α MAbs to achieve a better therapy. Clinical relevance of these strategies is discussed.

MATERIALS AND METHODS

Animals. Six- to eight-week-old female BALB/c scid/scid (SCID) mice and female BALB/c mice were purchased from Japan Clea (Tokyo, Japan) and maintained in a specific pathogen-free condition at Tokyo Medical and Dental University. All animal procedures in this study were performed according to the guidelines for animal experiments of Tokyo Medical and Dental University.

Induction of colitis and antibody treatment. Colitis was induced in SCID mice by adoptive transfer of CD4+CD45RBhigh T cells, essentially as described previously (48). CD4+ T cells were isolated from splenic mononuclear cell from BALB/c mice using the anti-CD4 (L3T4) MACS magnetic separation system (Miltenyi Biotec, Auburn, CA) according to the manufacturer’s instruction. Enriched CD4+ T cells were then labeled with phycoerythrin (PE)-conjugated anti-CD4 MAb (16A, PharMingen) and sorted into CD45RBhigh (highest staining 30%) and CD45RB low (lowest staining 4%) T cells. These mice were then administered intraperitoneally with 250 μg anti-OX40L MAb (RM134L, Rat IgG2b) (1) in 250 μl PBS three times per week, starting at the day of T cell transfer, over a period of 8 wk in the preventive protocol. An equivalent amount of control rat IgG MAb (MP6-XT22) was used as control. In another set of experiments, we treated another group of mice by intraperitoneal injection of 250 μg anti-OX40L MAb or control IgG three times per week, starting from 3 wk after T cell transfer, for 4 wk in the therapeutic protocol. In another set of experiments, we treated four groups of mice three times per week, from 3 to 8 wk after T cell transfer, with 1) 500 μg control IgG, 2) 250 μg anti-OX40L MAb + 250 μg control IgG, 3) 250 μg anti-TNF-α MAb (MP6-XT22) + 250 μg control IgG, or 4) 250 μg anti-OX40L MAb + 250 μg anti-TNF-α MAb. All mice were killed at 7–8 wk after T cell transfer for histological examination and preparation of lamina propria (LP) T cells.

Disease monitoring and clinical scoring. Mice were weighed and monitored for appearance and signs of soft stool and diarrhea weekly. Clinical score was assessed at 7–8 wk after T cell transfer as the sum of three parameters as follows: hunching and wasting, 0 or 1; colon thickening, 0–3 (0, no colon thickening; 1, mild thickening; 2, moderate thickening; 3, extensive thickening); and stool consistency, 0–3 (0, normal beaded stool; 1, soft stool; 2, diarrhea; 3, gross bloody stool).

Histological examination. Tissue samples were fixed in 6% phosphate-buffered formalin. Paraffin-embedded sections (5 μm) were stained with hematoxylin and eosin. Three tissue samples from the proximal, middle, and distal parts of the colon were prepared. The sections were analyzed without prior knowledge of the type of treatment. The mean degree of inflammation in the colon was calculated using a modification of a previously described scoring system (22).

Preparation of lamina propria lymphocytes and splenocytes. For the isolation of LP lymphocytes (LPL) from the colon, the entire length of intestine was opened longitudinally, washed with PBS, and cut into small pieces. The dissected mucosa was incubated two times with Ca2+−Mg2+-free Hanks’ balanced salt solution containing 1 mM dithiothreitol (Sigma) for 30 min each to remove mucus. The supernatants containing intraepithelial and epithelial cells were removed. Collected tissues were treated with 2 ml collagenase A (Worthington Biomedical, Freehold, NJ) and 0.01% DNase (Worthington) in RPMI1640 medium for 2 h. The cells were pelleted two times through a 40% isotonic Percoll solution and then further purified by Ficoll-Hypaque density gradient centrifugation (40%/75%). CD4+ LPL were obtained by positive selection using the anti-CD4 (L3T4) MACS magnetic separation system (Miltenyi Biotec). The cells were then 95% CD4+ when analyzed by flow cytometry. Splenic mononuclear cells were obtained from the same animals by mechanical dissociation of the spleen followed by Ficoll-Hypaque density gradient centrifugation.

Flow cytometry. Isolated LPL or splenocytes were preincubated with Fcγ receptor-blocking MAb (2.4G2) for 20 min, followed by incubation with FITC-, PE-, or biotin-labeled MAb for 30 min on ice. Biotinylated MAb was detected with PE-, PE-Cy7-, or Cy5-conjugated streptavidin (BD PharMingen). All reagents were obtained from PharMingen. Two-color flow cytometric analysis was performed on FACSscan (Becton-Dickinson) equipped with CellQuest software.

Cytokine ELISA. LP CD4+ T cells (1 X 105) were cultured in 200 μl of RPMI1640 medium supplemented with 10% FCS and 2 μg/ml anti-CD28 MAb (37.51, PharMingen) in 96-well plates (Costar, Cambridge, MA) precoated with 5 μg/ml anti-CD3e MAb (145–2C11, PharMingen) for 48 h. Cytokine concentrations in the culture supernatants were determined by specific ELISA according to the manufacturer’s instructions (R&D, Minneapolis, MN).

Statistical analysis. Significant differences between two groups were determined by Mann-Whitney U-test. P values <0.05 were considered to be statistically significant.

RESULTS

Administration of Anti-OX40L MAb prevents the development of colitis. We induced chronic colitis in BALB/c SCID mice by adoptive transfer of CD4+CD45RBhigh T cells from normal BALB/c mice. These mice manifested progressive weight loss from 3 wk after T cell transfer and clinical symptoms of colitis such as diarrhea with increased mucus in the stool, anorectal prolapse, and hunched posture by 6–8 wk.
The colons from these mice were enlarged and had greatly thickened walls due to severe colonic inflammation (data not shown).

To explore the contribution of OX40/OX40L interaction to the development of chronic colitis, a neutralizing anti-OX40L MAb or control rat IgG was administered to the recipient mice from the day of T cell transfer and then three times per week for 8 wk. As shown in Fig. 1A, the control IgG-treated mice manifested progressive weight loss (wasting disease) from 3 to 5 wk after T cell transfer. These mice had diarrhea with increased mucus in the stool, anorectal prolapse, and hunched posture by 6–8 wk. In contrast, the anti-OX40L MAb-treated mice appeared healthy, with a gradual increase in body weight and without diarrhea during the whole period of observation (Fig. 1A). At 8 wk after T cell transfer, the colon from the control IgG-treated mice but not that from the anti-OX40L MAb-treated mice, was enlarged and had a greatly thickened wall (Fig. 1B). In addition, the splenic enlargement was also present in the control IgG-treated mice compared with the anti-OX40L MAb-treated mice. Totally, the assessment of colitis by clinical scores showed a clear difference between the control IgG-treated mice and anti-OX40L MAb-treated mice (Fig. 1C). Histological examination showed prominent epithelial hyperplasia with glandular elongation with a massive infiltration of mononuclear cells in the lamina propria of the colon from the control IgG-treated mice (Fig. 1D). In contrast, the glandular elongation was mostly abrogated and only few mononuclear cells were observed in the lamina propria of the colon from anti-OX40L MAb-treated mice (Fig. 1D). This difference was also confirmed by histological scoring of multiple colon sections, which was 5.7 ± 1.2 in control rat IgG-treated mice vs. 0.8 ± 0.6 in anti-OX40 MAb-treated group (P < 0.005; Fig. 1E). A further quantitative evaluation of CD4+ T cell infiltration was made by isolating LPL from the resected bowels. Only a few CD4+ T cells were recovered from the colonic tissue of anti-OX40L MAb-treated mice compared with those from the control rat IgG-treated mice (Fig. 1F). The number of CD4+ cells recovered from the colon of control IgG-treated mice (42.1 ± 18.3 × 10^5) far exceeded the number of originally injected cells (5 × 10^5), indicating an extensive T cell proliferation in the inflamed colon, which was mostly abrogated in the anti-OX40L MAb-treated mice (4.7 ± 1.2 × 10^5). Furthermore, the number of CD4+ splenocytes from control IgG-treated mice was significantly increased as comparable to that from age-matched normal BALB/c mice. In contrast, the number of CD4+ splenocytes from anti-OX40L MAb-treated mice was significantly less than that from control IgG-treated mice (Fig. 1G).

We also examined the cytokine production by CD4+ LPL from the control IgG- or anti-OX40L MAb-treated mice. As shown in Fig. 2, CD4+ LPLs from the anti-OX40L MAb-treated mice produced significantly less APRIL, and the number of CD4+ LPLs was determined by histological scoring of multiple colon sections, which was 5.7 ± 1.2 in control rat IgG-treated mice vs. 0.8 ± 0.6 in anti-OX40 MAb-treated mice (P < 0.005; Fig. 1E). A further quantitative evaluation of CD4+ T cell infiltration was made by isolating LPL from the resected bowels. Only a few CD4+ T cells were recovered from the colonic tissue of anti-OX40L MAb-treated mice compared with those from the control rat IgG-treated mice (Fig. 1F). The number of CD4+ cells recovered from the colon of control IgG-treated mice (42.1 ± 18.3 × 10^5) far exceeded the number of originally injected cells (5 × 10^5), indicating an extensive T cell proliferation in the inflamed colon, which was mostly abrogated in the anti-OX40L MAb-treated mice (4.7 ± 1.2 × 10^5). Furthermore, the number of CD4+ splenocytes from control IgG-treated mice was significantly increased as comparable to that from age-matched normal BALB/c mice. In contrast, the number of CD4+ splenocytes from anti-OX40L MAb-treated mice was significantly less than that from control IgG-treated mice (Fig. 1G).

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IFN-γ, IL-2, and TNF-α compared with those from the control IgG-treated mice on in vitro stimulation. In contrast, the production of IL-4 or IL-10 was not significantly affected. These results suggested that anti-OX40L MAb prevented the development of colitis primarily by inhibiting the expansion and/or infiltration of pathogenic T cells in the colon and secondarily by inhibiting the development of pathogenic Th1 cells producing IFN-γ, IL-2, and TNF-α.

Therapeutic effect of anti-OX40L MAb. We next evaluated the therapeutic effect of anti-OX40L MAb treatment on the ongoing disease. Because the wasting disease started 3 wk after T cell transfer (Fig. 1A) and the infiltration of lymphocytes and colitis was already detectable at 2 wk (data not shown), we started the anti-OX40L MAb treatment from 3 wk after T cell transfer. As shown in Fig. 3A, the anti-OX40L MAb-treated mice exhibited a significant improvement of weight loss compared with the control IgG-treated mice. The anti-OX40L MAb-treated mice did not exhibit clinical manifestations such as diarrhea and hunched posture, which were substantiated by clinical scoring at 7 wk (Fig. 3B). Histological examination of the colon from anti-OX40L MAb-treated mice revealed significant reduction of granulomatous inflammation, leukocyte infiltration, and epithelial hyperplasia (Fig. 3C). Histological scores were significantly decreased in the anti-OX40L MAb-treated mice (2.1 ± 0.6) compared with the control IgG-treated mice (5.4 ± 1.8; P < 0.05; Fig. 3D). The number of CD4+ LPL was greatly reduced in the anti-OX40L MAb-treated mice (7.4 ± 0.2 × 10^5) compared with that from the control IgG-treated mice (28.1 ± 6.2 × 10^5; P < 0.01; Fig. 3E). Furthermore, the number of CD4+ MAb-treated mice was significantly reduced compared with that from control IgG-treated mice (Fig. 3F). These results indicated that the therapeutic administration of anti-OX40L MAb could inhibit the progression of ongoing colitis by suppressing the expansion and/or infiltration of pathogenic T cells in the colon.

Combination effect of anti-OX40L and anti-TNF-α MAbs. We finally examined the combined effect of anti-OX40L and anti-TNF-α MAbs. At 3 wk after T cell transfer, we started to treat the mice with control IgG, anti-OX40L MAb alone, anti-TNF-α MAb alone, or anti-OX40L and anti-TNF-α MAbs until 7 wk. Anti-TNF-α MAb alone, as well as anti-OX40L MAb alone, markedly improved the weight loss, and the combination of both MAbs results in further improvement (Fig. 4A). Ameliorating effects of anti-TNF-α MAb or anti-OX40L MAb alone and the additive effect of both MAbs were also observed in clinical scores (Fig. 4B). However, anti-TNF-α MAb alone exerted a rather weak or no significant effect on histopathological changes (Fig. 4, C and D) and CD4+ T cell expansion/infiltration in the colon (Fig. 4E). Nevertheless, the combination of anti-TNF-α MAb with anti-OX40L MAb resulted in significantly more improvement of histological score (Fig. 4E) and more reduction of CD4+ LPL and CD4+ splenocytes (Fig. 4, E and F) compared with anti-OX40L MAb alone.

In vitro stimulation of CD4+ LPL showed that combination of anti-TNF-α and anti-OX40L MAbs significantly reduced the production of IFN-γ, IL-2, and TNF-α compared with anti-OX40L MAb alone, whereas anti-TNF-α MAb alone only significantly inhibited the TNF-α production (Fig. 5). These results suggested that the combination with anti-TNF-α MAb could improve the therapeutic effect of anti-OX40L MAb by additively inhibiting the development of pathogenic Th1 cells producing IFN-γ, IL-2, and TNF-α.

DISCUSSION

In the present study, we demonstrate a possible contribution of OX40/OX40L interaction to the pathogenesis of the murine CD model by showing that administration of neutralizing anti-OX40L MAb effectively prevented the onset and progression of CD4+CD45RBhigh-transferred colitis and significantly abrogated infiltration of CD4+ T cells and local Th1 cytokine production in the inflamed colon. Our present results further substantiated the important role of the OX40-OX40L costimulatory pathway in CD4+ T cell-dependent inflammatory responses and tissue damage in intestinal mucosa. Because the expression of OX40...
OX40L interactions would be beneficial in colitis, suggesting that the blockade of OX40/OX40L still improved clinical and histological features of ongoing CD4 T cells, hinting that the anti-OX40L MAb may be a useful strategy for controlling a variety of T cell-mediated inflammatory diseases. Consistent with our present study, Malmstrom et al. (25) also recently reported that early administration of anti-OX40L MAb (OX89) starting at the time of disease induction prevented the development of CD4+CD45RBhigh-transferred colitis. In addition, we also showed that a delayed treatment with anti-OX40L (RM134L) still improved clinical and histological features of ongoing CD4+CD45RBhigh-transferred colitis, suggesting that the blockade of OX40/OX40L interactions would be beneficial for the treatment of human CD.

The treatment of CD depends largely on chronic use of immunosuppressive reagents that can result in severe side effects. In the last 5 years, a number of more specific targets for potential treatment have been discovered in murine colitis models. This has so far led to the introduction of an anti-TNF-α MAb (Infliximab) to the patients with CD, which has been effective in two-thirds of CD patients. Infliximab is a chimeric human/murine MAb of IgG1 isotype with the specificity for human TNF-α. The treatment with Infliximab has also been reported to induce monocytopenia rapidly after infusion and to profoundly downregulate monocytes in CD mucosa. Recently, it has been shown that Infliximab bound specifically to membrane-bound TNF-α, as well as TNFR-bound TNF-α, and also that Infliximab induced apoptosis in peripheral monocytes of patients with chronic active CD in a dose-dependent manner. Of particular clinical importance, we showed here that the delayed anti-OX40L treatment does not only ameliorate established colitis, but also induces a striking improvement in combination with anti-TNF-α MAb. However, anti-TNF-α MAb alone could not ameliorate established colitis in this study. Unlike Infliximab, the anti-mouse TNF-α MAb used in the present study might not be able to induce apoptosis of TNF-α-bearing cells, such as macrophages and dendritic cells, but just neutralize the proinflammatory action of TNF-α. Consistent with this notion, although the anti-TNF-α treatment alone could not suppress the intestinal inflammation, it improved wasting, which was caused by "cachectic" action of TNF-α.

The mode of OX40/OX40L contribution to intestinal inflammation still remains unclear. First, we found that OX40L was not expressed on CD4+ LPL from both normal and colitic mice (data not shown). This suggests that the therapeutic effect of our anti-OX40L (RM134L, rat IgG2b) is not due to the CD4+ T cell depletion by these monoclonal antibodies. In the intestinal environment, dendritic cells sampling intestinal antigens be-

Fig. 3. Therapeutic effect of anti-OX40L MAb. The recipient mice were administered with anti-OX40L MAb or control rat IgG for 4 wk from 3 wk after CD4+CD45RBhigh T cell transfer (arrow). A: change in body weight over time is expressed as %original weight. Data are represented as the means ± SE of 6 mice in each group. *P < 0.05 compared with control IgG. B: clinical scores were determined at 7 wk after T cell transfer. Data are indicated as the means ± SE of 6 mice in each group. *P < 0.05. C: histological examination of the colon from control IgG- or anti-OX40L-treated mice at 7 wk after T cell transfer. Original magnification, ×100. D: histological scores were determined at 7 wk after T cell transfer. Data are indicated as the means ± SE of 6 mice in each group, *P < 0.05. E: LPL were isolated from the colon at 7 wk after T cell transfer, and the number of CD4+ cells was determined by flow cytometry. Data are indicated as the means ± SE of 6 mice in each group. *P < 0.01. F: splenocytes were isolated at 7 wk after T cell transfer, and the number of CD4+ cells was determined by flow cytometry. Data are indicated as the means ± SE of 7 mice in each group. *P < 0.01.
come activated and migrate to the MLN, where they activate T cells to expand. Malmstrom et al. (25) have shown that OX40L was upregulated in local MLN but not in inflamed mucosa from colitic mice by immunohistochemical staining, suggesting that the OX40/OX40L interaction may be essential for the process of antigen presentation or effector cell expansion in MLN. Having the evidence that the delayed anti-OX40L treatment did ameliorate the established colitis, we prefer that OX40/OX40L interaction might be involved in the effector T cell expansion in the MLN. However, there are several reports indicating that activated macrophages and dendritic cells existed and might function in inflamed mucosa in this model, which might transiently express OX40L and contribute to activation of pathogenic T cells locally.

The functional role of OX40L vs. CD40, which is a similar member of the TNF family, in the activation of T cells should be mentioned. Previous studies have shown that anti-CD40L prevented the development of 2,4,6-trinitrobenzene sulfonic acid colitis but did not treat colitis (43). In subsequent studies, anti-CD40L was found to ameliorate several forms of colitis at least partially (8). The failure to treat colitis with anti-CD40L might be attributed to the possibility that after inflammation is initiated, APC can be activated to produce IL-12 by LPS and other factors. Furthermore, previous studies of OX40/OX40L interaction have suggested that the interaction may be necessary not for initial activation of APC, but rather for sustained activation (42). Thus, whereas CD40L induced IL-12 production, both CD40L and OX40 may be necessary for sustained IL-12 production and may be critical to IL-18 production. Together with our present data, anti-CD40L administration at the early phase and anti-OX40L administration at the late stage in the develop-

![Fig. 4. Additive effect of anti-TNF-α and anti-OX40L MAbs. The recipient mice were administered with control rat IgG, anti-OX40L MAb, anti-TNF-α MAb, or anti-TNF-α + anti-OX40L MAbs for 4 wk from 3 wk after CD4+CD45RBhigh T cell transfer (arrow). A: change in body weight over time is expressed as %original weight. Data are represented as the means ± SE of 7 mice in each group. *P < 0.05 compared with anti-TNF-α or anti-OX40L MAb alone. B: clinical scores were determined at 7 wk after T cell transfer. Data are represented as the means ± SE of 7 mice in each group. *P < 0.05 compared with control IgG. **P < 0.05 compared with anti-OX40L MAb alone. C: histological examination of the colon from control IgG, anti-OX40L MAb, anti-TNF-α MAb, or anti-TNF-α + anti-OX40L MAb-treated mice at 7 wk after T cell transfer. Original magnification, ×100. D: histological scores were determined at 7 wk after T cell transfer. Data are represented as the means ± SE of 7 mice in each group. *P < 0.05 compared with control IgG. **P < 0.05 compared with anti-OX40L MAb alone. E: LPL were isolated from the colon at 7 wk after T cell transfer, and the number of CD4+ cells was determined by flow cytometry. Data are represented as the means ± SE of 7 mice in each group. *P < 0.05 compared with control IgG. **P < 0.05 compared with anti-OX40L MAb alone. F: splenocytes were isolated at 7 wk after T cell transfer, and the number of CD4+ cells was determined by flow cytometry. Data are indicated as the means ± SE of 7 mice in each group. *P < 0.05 compared with control IgG. **P < 0.05 compared with anti-OX40L MAb alone.
Development of colitis might be the best combination therapy. Further study will be necessary to address this point.

Another possible mechanism for anti-OX40L MAb therapy is the prevention of recruitment of OX40+ T cells to sites of inflammation through OX40L expression on endothelial cells. In fact, it has been shown that cells from patients with adult T cell leukemia adhere to endothelial cells through OX40-OX40L interaction (15). In addition, in human inflammatory bowel disease, OX40L+ endothelial cells have been seen (41). Further studies will be required to address this possibility.

So far, accumulating evidence supports the concept that a dominance of either Th1 or Th2 is associated with distinct manifestations of autoimmune diseases, and some therapies that induce a shift from Th1 or Th2 may result in amelioration of diseases (17, 32, 38). In the case of collagen-induced arthritis and EAE models, an IFN-γ-mediated Th1-type response has been shown to be pathogenic, and an IL-4-mediated Th2 response has been shown to be protective (27, 28). It has been reported that the blockade of some costimulatory pathways ameliorated EAE by deviating the pathogenic Th1 response toward the protective Th2 response (18, 21). How do OX40L molecules function in chronic intestinal inflammation? In this study, the blockade of OX40-OX40L interaction did not augment the Th2 response, because in vivo treatment with anti-OX40L MAb did not enhance either IL-4 or IL-10 production. In contrast, anti-OX40L MAb treatment significantly inhibited the IFN-γ production by lamina propria CD4+ T cells in inflamed mucosa. This suggests that the ameliorating effect of anti-OX40L MAb on the CD4+CD45RBhigh-transferred colitis model might be, at least in part, mediated by its inhibitory effect on the development of pathogenic Th1 cells producing IFN-γ. Consistent with this notion, it has been reported that the IFN-γ production was most dramatically affected by the absence of OX40 on T cells or the absence of OX40L on antigen-presenting cells in OX40- or OX40L-deficient mice (5, 20, 35). Another possible mechanism for the ameliorating effect of anti-OX40L MAb on the CD4+CD45RBhigh.
transferred colitis model may involve its inhibitory effect on the recruitment of OX40+ T cells to the inflamed mucosa, because the expression of OX40L on endothelial cells has been implicated in their interaction with activated T cells expressing OX40 (29, 33). Further studies are needed to elucidate the exact mechanism for the prevention of colitis by the blockade of OX40/OX40L interaction.

In summary, our present findings suggest that regulation of the OX40/OX40L pathway may be of key importance in successful treatment of CD and also that anti-OX40L in combination with anti-TNF-α therapy may be useful for refractory CD.

We thank Drs. W. Strober and M. Azuma for critically reading the manuscript and H. Fujimoto and H. Nishikawa for technical assistance. This work was supported, in part, by grants-in-aid from the Japanese Ministry of Education, Culture, and Science and the Japanese Ministry of Health and Welfare.

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