Usefulness of CD4⁺CD45RB<sup>high</sup>CD25⁻ Cell–Transferred SCID Mice for Preclinical Evaluation of Drugs for Inflammatory Bowel Disease

Daisuke Hirano<sup>1,2,*</sup> and Shihoko Kudo<sup>3</sup>

<sup>1</sup>Research and Development Division, Perseus Proteomics, Inc., 4-7-6 Komaba, Meguro-ku, Tokyo 153-0041, Japan
<sup>2</sup>Life Science Research Laboratories, FUJIFILM Corporation, 577, Ushijima, Kaisei-machi, Ashigarakami-gun, Kanagawa 258-8577, Japan
<sup>3</sup>UMN Pharma, Inc., Exploratory Research Division Molecular Medicine Laboratory, Bioscience Education and Research Center, Akita University, 1-1-1 Hondo, Akita 010-8543, Japan

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Abstract. Mouse colitis induced by transfer of CD4⁺CD45RB<sup>high</sup>CD25⁻ cells share many pathological features with human inflammatory bowel disease (IBD). However, there is little known about how mouse colitis responds to drugs used for IBD treatment. To address this issue, we have investigated the effects of the IBD drugs, dexamethasone and anti-tumor necrosis factor-α antibody, on the mouse experimental colitis. Administration of either drug ameliorated their morbid signs such as body weight loss, colon shortening, and an increased ratio between colon and body weights (C/B ratio). Also improved were mucosal inflammatory signs in the colon, and histological damage scores were significantly decreased. Of the proinflammatory cytokines assayed in colon and plasma samples from the colitis mice, the colonic interleukin (IL)-1β level alone was significantly decreased by either drug administration. Regression analysis of data obtained with either drug revealed a close correlation between the histological damage score and C/B ratio or colonic IL-1β level. The present results show that the experimental mouse colitis responds to IBD drugs with its amelioration and that the C/B ratio and colonic IL-1β are available as a disease marker for IBD, suggesting the usefulness of this mouse model of colitis for pre-clinical screening of drug candidates for IBD treatment.

Keywords: inflammatory bowel disease, experimental model, SCID mice, corticosteroid, anti-tumor necrosis factor (TNF)-α antibody

Introduction

A variety of chronic inflammatory diseases occur in the gastrointestinal tract. The most enigmatic is idiopathic inflammatory bowel disease (IBD), which encompasses at least two forms of disease: Crohn’s disease and ulcerative colitis. Key clinical features of these diseases include abdominal pain, body weight loss, diarrhea, and rectal bleeding, although some certain pathological differences are found between them. Crohn’s disease may affect every portion of the gastrointestinal tract, in which inflammation extends intramurally in the bowel wall, resulting in formation of granulomas and fistula (1). On the other hand, the affected region of ulcerative colitis is restricted to the colon and rectum, and inflammation occurs in the inner lining of the gut, especially in the mucosa and submucosa, with formation of crypt abscesses and ulceration there (1). Despite the long-standing clinical entities of these diseases, their exact etiology remains uncertain. Studies with human IBD patients have shown that genetic and environmental factors are both involved in the pathogenesis of IBD (2). There has also been evidence indicating that IBD is a consequence of malfunction of the gut mucosal immune system, possibly due to functional abnormality of commensal gut bacteria (2, 3), but also that it is a T-cell–mediated disease in which specific subsets of T cells are involved (4).

Many animal models for studying the pathogenic
mechanism of IBD have been developed (4, 5). Their use has provided novel, relevant insights into understanding of its pathogenesis, especially in terms of cell subsets and various biochemical factors including cytokines, chemokines, and cell adhesion molecules. An experimental colitis in severe combined immunodeficient (SCID) mice that lack functional T and B cells is generally taken as a most promising animal model of IBD. The experimental colitis is induced after transfer of a naïve CD4+ T cell-subset expressing high level of CD45RB (CD45RB<sup>high</sup>) into the mice (6, 7). The induction of colitis can be prevented by concomitant transfer of CD45RB<sup>low</sup> cells, which are thought to be activated/memory T cells (7), and thus it seems likely to be a T-cell–mediated disease. The colitis may also involve commensal gut bacteria in its development since the CD4<sup>+</sup>CD45RB<sup>high</sup> cell–transferred mice were not affected by colitis when raised under germ-free conditions (8, 9). Furthermore, the cell-transferred colitis mice exhibit many of the clinical and histological features of IBD such as diarrhea, body weight loss, thickened mucosa, crypt abscesses, and mucosal inflammation with infiltration of macrophages, lymphocytes, and neutrophils. The mucosal inflammation is suggested to be due to a Th-1–mediated response involving interleukin (IL)-12 and interferon (IFN)-γ (10, 11), as deduced in the case for IBD. In these ways, there is substantial similarity between the experimental colitis in this model and human IBD.

However, little is yet known about how drugs used for the treatment of IBD affect this experimental colitis, so it is unclear whether this disease model is applicable to preclinical evaluation of drug candidates for IBD therapy. To address these issues, we have investigated the effects of dexamethasone and anti-tumor necrosis factor (TNF)-α antibody, both widely used for IBD treatment, on some certain clinical, histological, and biochemical signs that may be associated with the mouse colitis.

**Materials and Methods**

**Animals**

All procedures described below were performed following the protocol approved by the animal ethics committee of Perseus Proteomics, Inc. according to the guidelines for proper conduct of animal experiments (Science Council of Japan). BALB/c and C.B.-17 SCID mice were purchased from CLEA Japan (Tokyo). The mice were maintained in specific pathogen–free conditions and fed autoclaved food and water ad libitum. The animals used in the present study were female and between 10 and 11 weeks of age.

**Agents**

FITC-conjugated rat anti-mouse CD4 (L3T4) monoclonal antibody (clone GK 1.5), PE-conjugated rat anti-mouse CD45RB monoclonal antibody (clone 16A), biotin-conjugated rat anti-mouse CD25 (IL-2 receptor alpha chain, p55) monoclonal antibody (clone PC61), and propidium iodide (PI) solution (50 mg/ml) were purchased from BD Biosciences (San Jose, CA, USA). Rat anti-TNF-α antibody (clone MPG-XT3) was purchased from Upstate biotechnology (Lake Placid, NY, USA) and dexamethasone, from Sigma-Aldrich (St. Louis, MO, USA).

**Preparation of CD4<sup>+</sup>CD45RB<sup>high</sup> CD25<sup>−</sup> cells and induction of colitis**

Cells used to induce colitis were prepared according to the method of Morrissey et al. (6) with some slight modifications. Spleens and mesenteric lymph nodes were dissected from BALB/c mice and teased into a cell suspension. After washing the cells, CD4<sup>+</sup>CD25<sup>−</sup> cells were enriched using a CD4<sup>+</sup> cell isolation kit (Miltenyi Biotec, Germany) and biotinylated anti-CD25 monoclonal antibody according to the instruction manual. Then the cells were stained with FITC-conjugated anti-CD4 and PE-conjugated anti-CD45RB monoclonal antibodies and PI, followed by purification with a flow cytometry. Cell sorting was performed on a FACS Vantage SE<sup>®</sup> with TurboSort<sup>®</sup> option (Becton Dickinson & Co., Franklin Lakes, NJ, USA) at an analysis rate of 7500 cells/sec under 30 PSI of sheath pressure. CD4<sup>+</sup>CD45RB<sup>high</sup> CD25<sup>−</sup> cells were sorted as the brightest 45% of the CD4-positive and PI-negative population. The sorted cells were washed and resuspended at 1.5 × 10<sup>6</sup>/ml in cold PBS. The cell suspension prepared in these ways was injected intraperitoneally in a volume of 200 μl into C.B.-17 SCID mice. For comparison, the same volume of PBS was similarly injected into another group of the same strain mice (which will be referred to as non-cell transfer mice). These groups of mice were individually marked and weekly weighed. The body weight data from each mouse were expressed as the percentage relative to its body weight on the day of cell or PBS injection (initial body weight). The mice were kept for 4 weeks and those that had relative body weights in the range of the mean ± S.D. were selected to minimize potential data dispersion for evaluation of drug efficacy.

**Treatment with dexamethasone or anti-TNF-α antibody**

The cell-transferred mice selected were divided into multiple groups (n = 6 – 8 for each) with the individual groups having a comparable mean value for the relative body weight.
Dexamethasone (0.1, 0.3, and 1.0 mg/kg, s.c.; 100 μl for each) was administered every day for 14 days (day 0–day 13), with one group being treated with one dose level. Anti-TNF-α antibody or the isotypical antibody IgG1 (100 μg in 100 μl of PBS, i.p.) was administered at day 0, 5, and 10 during a period of 14 days (day 0–day 13). As control, a vehicle (saline or PBS, 100 μl) was administrated to some other groups of colitic mice (control groups) in the same protocol as described above for the respective drugs. The vehicle was also similarly administrated to non-cell transfer mice (n = 6).

**Colonic tissue and plasma sampling**

On the day 14 of each drug test, the blood was collected under anesthesia from the drug-treated and control colitic mice and non-cell transfer mice by using a heparinized syringe. It was then centrifuged at 3000 × g for 5 min at 4°C, and the supernatants (plasma) were stored at −80°C until they were assayed for plasma cytokines. Following the blood collection, the colon was removed over the whole length, opened longitudinally, cleared of fecal material with 0.9% saline solution, and blotted dry on a paper towel. The whole length of the gut tissue was measured with no forcible stretch. Also, its wet weight was measured to determine the ratio between the colon and body weights (C/B ratio), which is taken as a reliable index of colonic inflammation (12, 13). After that, the gut tissue was divided in half longitudinally; one half was used for histological analysis and the other half was frozen in liquid N₂ and stored at −80°C until preparation for the cytokine assay (see below).

**Histological analysis and determination of cytokine levels**

The half-divided colon tissue was fixed in 4% phosphate-buffered formalin solution and divided into the proximal, middle, and distal parts. They were then embedded in paraffin, cut into tissue sections, and stained with hematoxylin and eosin. The sections stained were inspected microscopically, and histological damages of the individual parts were scored on a scale of 0 = absent to 4 = severe as described by Simon et al. (14). The histological damage score for each mouse was given as the sum of the scores in the three parts (from 0 up to 12 in total).

**Determination of cytokine levels in plasma and tissue by ELISA**

The frozen colon tissues were homogenized in ice-cold PBS (pH 7.2) containing a protease inhibitor cocktail (Sigma-Aldrich). Homogenates were then centrifuged at 15,000 × g for 5 min at 4°C and the supernatants were stored at −80°C until the cytokine assay. Colonic tissue and plasma levels of cytokines such as IL-1β, IL-12, IL-6, and TNF-α were determined with the Quantikine ELISA kit (R&D systems, Minneapolis, MN, USA) according to the instruction manuals. The cytokine levels in the colon tissue were expressed as picograms per 100 mg tissue wet weight.

**Statistical analyses**

Values in the text are given as the means ± S.E.M. All statistical analyses were performed using JMP software version 7.0 (SAS Institute, Cary, NC, USA). Parametric data were analyzed by one-way ANOVA followed by Dunnett’s multiple comparisons post test or unpaired Student’s t-test, and nonparametric data (histological damage score) were analyzed with the Mann-Whitney’s U test. Relationships between the histological damage score and each of the other morbid signs were investigated by single regression analysis. When P value was <0.05, differences were considered statistically significant.

**Results**

On day 14 of dexamethasone or anti-TNF-α antibody (or vehicle) treatment, various morbid signs and cytokine levels were determined in drug-treated and vehicle-treated (control) groups of colitis mice and in the vehicle-treated group of non-cell transfer mice (normal group). Every factor determined was considered as described above for the respective groups (6, 7). Effects of the drug treatment were evaluated by comparing data between the drug-treated and control groups.

**Dexamethasone ameliorates colonic inflammation in CD4+CD45RBhigh CD25− cell–transferred SCID mice**

The control group of mice had a relative body weight of 91.8 ± 4.0% (n = 7), significantly smaller than that of the normal group (111.4 ± 1.1%, n = 6), indicating a clear loss of body weight caused by colitis in the former group. Dexamethasone reduced the body weight loss, as the drug-treated groups had relative body weights of 96.6 ± 2.5%, 98.0 ± 1.8%, and 97.1 ± 1.7% (n = 7, 7, and 8, respectively) at the respective doses of 0.1, 0.3, and 1.0 mg/kg. However, each mean value did not significantly differ from the control value (Fig. 1A).

As shown in Fig. 1B, the colon length of the control group (9.0 ± 0.4 cm) was shorter than that of the normal group (10.8 ± 0.3 cm). The drug-treated group had a significantly longer colon length than the control value.
at any of the three doses (10.1 ± 0.5 cm at 0.1 mg/kg, 10.3 ± 0.4 cm at 0.3 mg/kg, and 11.0 ± 0.5 cm at 1.0 mg/kg). The colon length at 1.0 mg/kg was almost the same as that in the normal group (see above), indicating a complete restoration from the gut shortening due to colitis.

As for the C/B ratio, compared with the normal group (0.94 ± 0.03), the control group had an increased value of 2.53 ± 0.24 (Fig. 1C). Dexamethasone dose-dependently reduced the increase in the C/B ratio, as the drug-treated groups had the C/B ratios of 2.12 ± 0.15, 1.90 ± 0.15, and 1.59 ± 0.10 at the respective doses of 0.1, 0.3 and 1.0 mg/kg.

Histological observation of colonic sections from the control group revealed severe colitis characterized by colonic epithelial cell hyperplasia, leukocyte infiltration into both the mucosa and submucosa, and depletion of mucin-secreting goblet cells (Fig. 2B). No such inflammatory damages were seen in the colon of the normal group (Fig. 2A). As can be seen from Fig. 2C, treatment with dexamethasone at 1.0 mg/kg resulted in amelioration of the inflammatory damages. Consistently, dexamethasone dose-dependently reduced the histological damage score with a significant difference at 1.0 mg/kg from the control value (Fig. 2D).

The proinflammatory cytokines IL-1β, IL-12, IL-6, and TNF-α were assayed in colonic tissue and plasma samples. The individual cytokine levels were extremely elevated in the control group in comparison with the normal group (see open and closed columns in Fig. 3). As shown in Fig. 3A, dexamethasone treatment resulted in a dose-dependent reduction in the colonic level of IL-1β. The colonic IL-1β levels at 0.3 and 1.0 mg/kg (4800 ± 1700 and 3100 ± 1200 pg/100 mg tissue, respectively) each significantly differed from the control value (13500 ± 2300 pg/100 mg tissue). On the other hand, the plasma IL-1β levels at any doses tested did not significantly differ from the corresponding control value (5.63 ± 1.36 pg/ml, Fig. 3B). Plasma IL-12 level was reduced by dexamethasone treatment in a dose-dependent manner and the effect was statistically significant. The reduction of colonic IL-12 level was not significant, although it showed a dose-dependent tendency (Fig. 3: C and D). Colonic and plasma levels of IL-6 in the control group (100.0 ± 56.7 pg/100 mg tissue and 48.5 ± 22.6 pg/ml, respectively; data not shown) were relatively high compared with those in the normal group (8.8 ± 0.6 pg/100 mg tissue and 6.0 ± 1.1 pg/ml, respectively; data not shown). However, as can be seen from the S.E.M.s for the control values, there were considerable variations among the different control mice, so evaluation of drug effects on the cytokine levels was abandoned. The colonic TNF-α level remained almost

Fig. 1. Dexamethasone attenuates disease associated colon length shortening and increases in C/B ratio but not body weight loss in CD4+CD45RB<sup>high</sup> CD25<sup>−</sup> cell–transferred colitic mice. Body weights (A), colon lengths (B), and C/B ratios (C) in mice treated with dexamethasone at the three indicated doses (hatched columns) and with a vehicle (control: closed column) and in non-cell transfer mice (open column). The body weight of each mouse was expressed as % of that at the day of cell transfer (initial body weight), and its C/B ratio was expressed as the relative value of the colon weight to the body weight. Each column represents a mean ± S.E.M. (n = 6 – 8). * P<0.05 and ** P<0.01: significantly different from the control value. See the text for details.
unchanged with dexamethasone treatment at the three different doses (Fig. 3E). The plasma TNF-α could not be detected in any samples from the drug-treated, control, and normal groups.

Anti-TNF-α antibody also ameliorates colonic inflammation in CD4+CD45RBhigh CD25− cell–transferred SCID mice

In this series of tests, effects of an isotypical antibody (rat IgG1) were also investigated for comparison. In addition, a different control group (colitic mice without antibody treatment) as well as a different non-cell transfer (normal) group was used for these tests (see Material and Methods).

The control group had a decreased value for the relative body weight (89.2 ± 3.0%, n = 8) in comparison with the normal group (112.8 ± 1.4%, n = 6). The body weight loss was ameliorated by anti-TNF-α antibody treatment, as the antibody-treated group (94.9 ± 2.6%, n = 8) weighed significantly more than the control group did (Fig. 4A). The relative body weight in the isotypical treatment group (91.1 ± 2.5%, n = 8) did not significantly differ from the control value.

Similarly, the shortening of colon length (8.3 ± 0.3 cm in the control vs. 10.2 ± 0.4 cm in the normal group) was ameliorated by anti-TNF-α antibody treatment. The colon length in the anti-TNF-α antibody–treated group (9.7 ± 0.1 cm) was rather close to that in the normal
The isotypical treatment had no significant effect on the colon shortening (Fig. 4B). The control value for the C/B ratio had an increased value of 2.42 ± 0.09 compared with the normal group (1.00 ± 0.05). Anti-TNF-α antibody reduced the increased C/B ratio to 2.04 ± 0.10 with a significant difference, whereas the isotypical antibody almost had no effect (Fig. 4C).

Histological analysis in colonic sections from the control group revealed inflammatory damages (Fig. 5: A and B), as mentioned for the dexamethasone effects. The damages were ameliorated by anti-TNF-α antibody treatment (Fig. 5C). Consistently, the histological damage score in the antibody-treated group was significantly smaller than the control value. The corresponding value for the isotypical treatment group was almost the same as the control value (Fig. 5D).

Anti-TNF-α antibody treatment decreased the colonic
level of IL-1β from 13,400 ± 1500 (the control value) to 4600 ± 1000 pg/100 mg tissue with a statistical significance (Fig. 6A), but it hardly had any significant effect on the plasma IL-1β level (Fig. 6B). The isotypical antibody was almost without effect on the level of either cytokine (Fig. 6: A and B). Antibody treatment also almost had no effect on the colonic and plasma IL-12 levels (control values), which were increased in comparison with those in the normal group (Fig. 6: C and D). Anti-TNF-α antibody, but not the isotypical antibody, treatment significantly increased the colonic TNF-α level from 55.4 ± 2.8 (control value) to 81.3 ± 8.3 pg/100 mg tissue (Fig. 6E).

**Regression analyses**

Using the data obtained from the individual cell-transferred mice in the dexamethasone and anti-TNF-α antibody test, we explored the correlation between changes in the histological damage score, a direct morbid index, and in the other morbid signs examined. Figure 7A shows the correlation coefficients estimated from the data in the dexamethasone test (closed circles) and the anti-TNF-α antibody test (open circles). In both drug tests, the C/B ratio or colonic IL-1β level was closely correlated with the histological damage score. Actually, the correlation coefficients for the C/B ratio were 0.73 and 0.79 (P < 0.05 for each) in the dexamethasone and anti-TNF-α antibody tests, respectively; and similarly those for the colonic IL-1β level were 0.81 and 0.79, respectively (P < 0.05 for each). Statistically significant correlations were observed between colon length and the histological damage score in both the dexamethasone and anti-TNF-α antibody test (−0.82 and −0.59, P < 0.05 for each), but the latter was somewhat smaller compared to those of the C/B ratio and colonic IL-1β level. Correlation coefficients for the body weight, colonic IL-12, and colonic TNF-α level were considerably varied between the two drug tests (−0.55 and −0.19, 0.59 and 0.39, and 0.69 and −0.08, respectively; Fig. 7A), and their correlation with the histological damage score were statistically significant only in the dexamethasone test. Plasma IL-1β levels in both the dexamethasone and anti-TNF-α antibody tests did not correlate with the histological damage score (P > 0.05). Figure 7B shows plots of the C/B ratio (y) against the histological damage score (x). The plots of data from the dexamethasone and anti-TNF-α antibody tests gave regression lines expressed by y = 0.14x + 0.87 and y = 0.14x + 0.90, respectively. Similar plots of the log [colonic IL-1β level] (y) against the histological damage score (x) (Fig. 7C) gave the regression lines of y = 0.16x + 2.32 and y = 0.12x + 2.68, respectively. There was no statistical difference in the regression
coefficient between each pair of equations (0.14 vs. 0.14 and 0.16 vs. 0.12; \( P < 0.05 \)). The results from statistical analyses of the experimental data indicate that regardless of the drug used, the change in the C/B ratio or colonic IL-1\(\beta\) level closely correlate with those in the histological damage score.

**Discussion**

Many animal disease models have been developed to study the etiology and pathogenic mechanisms of human IBD (4, 5). When transferred with CD4\(^{+}\)CD45RB\(^{high}\) cells, SCID mice are affected by colitis that is similar to human IBD in a number of clinical, histological, and biochemical features (6, 7). We have investigated effects of the IBD drugs, dexamethasone and anti-TNF-\(\alpha\) antibody, on various morbid signs associated with the experimental colitis in CD4\(^{+}\)CD45RB\(^{high}\) CD25\(^{-}\) cell–transferred SCID mice. The morbid signs checked were body weight loss, shortening of colon length, increases in C/B ratio, histological damage of the colon, and elevations of colon and plasma levels of cytokines such as IL-1\(\beta\), IL-12, and TNF-\(\alpha\). The present results have shown that these IBD drugs are effective enough to reduce the morbid signs as to the colon length, C/B ratio, colon histology, and colonic IL-1\(\beta\) level, supporting the idea that the mouse colitis responds to IBD drugs with its amelioration.

In general, the histological damage score in the colon is a direct index that reflects the severity of colitis in any type of IBD animal model. Our regression analysis revealed that the C/B ratio has a close correlation...
with the histological damage score in the data obtained with dexamethasone and anti-TNF-α antibody (Fig. 7); actually, the correlation coefficients for the data from these respective drug tests were 0.73 and 0.79, respectively. Scoring of histological damages usually requires much time and labor, but a keen power of observation is also needed as well as an objective eye. Then based on the close correlation between the C/B ratio and histological damage score, the former index that can be determined from only both colon and body weights is suggested to be not only useful but also more readily applicable for evaluation of colitis severity.

Shortening of the colon has been taken as a useful index to determine the severity of mouse colitis, especially that induced by dextran sulfate sodium (15). The view may hold true for the present experimental colitis. However, the correlation coefficients estimated between the colon length and histological damage score (−0.82

**Fig. 6.** Anti-TNF-α antibody suppresses increased levels of colonic IL-1β in CD4+CD45RBh−CD25− cell-transferred colitic mice. The colonic and plasma level of IL-1β (A, B) and IL-12 (C, D) and the colonic levels of TNF-α (E) were determined in non-cell transfer mice (normal: open columns) and cell-transferred, colitic mice treated with vehicle (control: closed columns), anti-TNF-α antibody (hatched columns), or isotypical antibody, rat IgG1 (dotted columns). Each column represents a mean ± S.E.M. (n = 6 – 8). **P<0.01: significantly different from the control value.
for the dexamethasone data and −0.59 for the anti-TNF-α antibody) were somewhat smaller than the corresponding correlation coefficients for the C/B ratio (see above). The difference might be caused by the difficulty to maintain appropriate conditions under which the full length of the excised colon was measured.

Either treatment with dexamethasone and anti-TNF-α antibody ameliorated body weight loss in the colitis mice, but neither exerted a statistically significant effect (Figs. 1A and 4A). Moreover, there was no significant difference among the effects of dexamethasone treatment at the three different doses used with a ten-fold range. Exact explanations for these observations are not known. A possibility is that although some certain morbid signs were significantly ameliorated by the drug treatment as mentioned above, body weight gain requires greater improvements in food intake and/or other important factors.

CD45 is a general marker of lymphocytes, and its isotype CD45RB is recognized as a marker to distinguish...
the naive T cells (CD45RB<sup>high</sup>) from the mature T cells (CD45RB<sup>low</sup>) (16). Because colitis in this model does not occur when recipients are maintained in germ-free conditions or when they have reduced bacterial loads (8, 9), it is thought that the pathogenesis of disease in this model is due to an unrestrained T helper 1 (Th1)-type response of naive T cells to the intestinal flora, which is normally downregulated by regulatory T cell subsets. Once Th1 cells are activated by luminal or minor self-antigens, they secrete interferon γ (IFN-γ), which in turn stimulates antigen-presenting cells to secrete inflammatory cytokines including IL-1β, TNF-α, and IL-12.

IL-1β and TNF-α share a multitude of proinflammatory properties and appear to be crucial to the amplification of mucosal inflammation of IBD (17–20). Both cytokines are secreted by monocytes and macrophages upon activation and induce intestinal macrophages, neutrophils, fibroblasts, and smooth-muscle cells to produce prostaglandins, proteases, and other soluble mediators of inflammation and injury, as well as other inflammatory and chemotactic cytokines. In addition, together with IL-6, they may contribute to the constitutional symptoms of IBD and lead to the generation of acute-phase protein (17). It has been reported that various cytokines including IL-1β, IL-12, IL-6, and TNF-α are involved in the pathogenesis of IBD (21–25). Increases in these cytokines in colonic tissues or plasma were also observed in this study. Considering the above observations, IL-1β and TNF-α are thought to play key roles in the pathogenesis of colitis in this model.

It is noted that the colonic IL-1β level was significantly reduced by treatment with either dexamethasone or anti-TNF-α antibody (Figs. 3A and 6A). Regression analysis indicated that colonic IL-1β level correlated well with the histological damage score (r = 0.81 and 0.79, in dexamethasone and anti-TNF-α antibody tests, respectively). On the other hand, both colonic level and plasma TNF-α level were hardly decreased in dexamethasone and anti-TNF-α antibody tests and did not show any correlation with the histological damage score except for the colonic TNF-α level in the dexamethasone test. Considering that anti-TNF-α antibody exerted significant therapeutic effects on the colitis in this model, it seems to be an inexplicable result.

One of the probable explanations for this discrepancy is the difference in mechanism of action between dexamethasone and anti-TNF-α antibody. Dexamethasone is one of the glucocorticoids, which have many mechanisms of action. The anti-inflammatory and immune-modulating effects of glucocorticoids both in patients with IBD and animal models of colitis have been studied to a limited extent. To the best of our knowledge, there has been no study on the effect of glucocorticoids on the colitis in this model. In patients with active Crohn’s disease, prednisolone, but not budesonide, inhibits neutrophil and peripheral blood lymphocyte expression of activation markers and TNF-α, although budesonide is effective in the treatment of Crohn’s disease. (26). Given that dexamethasone and budesonide share some sort of mechanism of action in common, it is not surprising that dexamethasone exerts an anti-inflammatory effect on colitis without a decrease in TNF-α expression.

Another enigma is a significant elevation of the colon TNF-α level by anti-TNF-α antibody treatment, opposite to a simple expectation. A possible explanation is that the formation of a complex of TNF-α and anti-TNF-α antibody might prevent TNF-α from being degraded by proteolytic enzymes in the colonic tissue. Then, although colonic TNF-α level in mice treated with anti-TNF-α antibody apparently seemed higher than that in mice treated with isotypic antibody, TNF-α in colonic tissue might have only low bioactivity in mice treated with anti-TNF-α antibody. Actually, such a phenomenon was reported by Mihara et al. who found a prolongation of IL-6 half-life when the treatment with anti IL-6 antibody was done (27).

The remaining question is why did only colonic IL-1β show a good correlation with the histological damage score, although TNF-α is also thought to play key roles in the pathogenesis of colitis in this model. We think that these two cytokines play slightly different roles. In IBD patients, treatment with anti-TNF-α therapy, infliximab, is efficacious in the majority of the patients (28) and also reduces recurrence (29). On the other hand, IL-1β has been implicated as a primary target for therapeutic intervention for the treatment of IBD (30), but its clinical applications have failed to yield results so far. Given that TNF-α is an earlier mediator than IL-1β and its effect is amplified in downstream pathways of colitis in this model, the variation of downstream mediator levels may better reflect the severity of inflammation than that of TNF-α. Actually, in the present study, the magnitude of increases in IL-1β and IL-12 induced by colitis in colons were much bigger than that of TNF-α (Figs. 3 and 6).

Taken together, we propose that the colonic IL-1β is a useful biochemical factor to measure the severity of the present experimental colitis. Further studies must be performed to determine if this factor also is a reliable parameter to determine the effects of other drugs used for IBD treatment.

It has been suggested that IL-1β stimulates production of various cytokines including IL-6 and IL-12 via activating macrophages (31). Our results can not be
explained solely by this scheme, since both colonic and plasma levels of IL-6 varied so much among control colitis mice that drug tests could not be meaningfully performed, and either the colonic level or plasma IL-12 level was little affected by dexamethasone or anti-TNF-α antibody treatment. How these cytokines were elevated in the colitis mice is unknown at present.

The present study was performed with a consideration of using mice optimal for drug tests that had been selected depending on their body weight 4 weeks after cell transfer (see Materials and Methods). Without such a consideration, variations of data from the drug test would be more enlarged, which would hinder reliable evaluation of drug efficacy. This consideration of body weight would help the present experiments more clearly show drug efficacy, compared with our preliminary experiments. Although a simple comparison should not be made, because of different morbid signs examined and dose regimen, the effect of anti-TNF-α antibody on colitis seems more obvious in the present study than in the previously reported one (10), in which the treatment with anti-TNF-α antibody during the first 4 weeks following cell transfer had little effect, but continued treatment reduced the severity of colitis compared with untreated control animals. The apparent difference might involve the previous selection of colitis mice optimal for the drug tests.

In conclusion, the present results demonstrate that the CD4+CD45RBhigh CD25+ cell–transferred mouse colitis is treatable with two representative IBD drugs, dexamethasone and anti-TNF-α antibody, and thus can be a useful model for preclinical evaluation of drug candidates for IBD therapy as well as pharmacological study of this disease. The present study, especially, provides the first evidence that glucocorticoids and anti-TNF-α antibody are efficacious on the colitis in this model using objective and measurable biochemical markers. Most importantly, our results strongly showed the possibility that the C/B ratio and the colonic tissue level of IL-1β are quantitative indices in the CD4+CD45RBhigh CD25+ cell–transferred SCID model, which serve reliably for drug evaluation.

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