CD4 T cells monospecific to ovalbumin produced by *Escherichia coli* can induce colitis upon transfer to BALB/c and SCID mice

Masaru Yoshida¹,², Tomohiro Watanabe¹,², Takashi Usui¹, Yoichi Matsunaga¹, Yasuhiro Shirai¹, Masashi Yamori¹, Toshiyuki Itoh¹,², Sonoko Habu³, Tsutomu Chiba², Toru Kita¹ and Yoshio Wakatsuki¹

Divisions of ¹Clinical Bio-regulatory Science, and ²Gastroenterology and Hepatology, Graduate School of Medicine, Kyoto University, 54 Shogo-in, Kawara-cho, Sakyo-ku, Kyoto 606-8507, Japan
³Division of Host Defense Mechanism, Department of Immunology, Tokai University School of Medicine, Kanagawa 259–1193, Japan

Keywords: animal model, bacterial flora, Crohn’s disease, cytokines, inflammatory bowel disease, mucosa, transgenic mice, ulcerative colitis

Abstract

Although some animal models suggest an involvement of CD4 T cells reactive to luminal microrbial antigen(s) for the pathogenesis of inflammatory bowel diseases (IBD), direct linkage between microflora-driven clonal expansion of CD4 T cells and the development of colitis has not been well studied. Here, BALB/c and SCID mice were given CD4 T cells purified from Rag-2⁻/⁻ mice crossed to transgenic mice expressing TCR specific to ovalbumin (OVA) then administered with antibiotic-resistant *Escherichia coli* producing OVA (ECOVA) or LacZ (ECLacZ) via the rectum. The ECOVA-inoculated BALB/c and SCID mice developed a subacute colitis with microscopic features of distortion of crypt architecture, loss of goblet cells, and focal infiltration by mononuclear cells in the lamina propria (LP) and submucosa. Expanding OVA-specific CD4 T cells were detected in colonic follicles of mice with ECOVA. Early in colitis, OVA-specific CD4 T cells producing IFN-γ predominate in the LP of the colon, which was followed by an emergence of OVA-specific CD4 T cells producing IL-4 and IL-10 at a later time point. Co-transfer of an IL-10-secreting OVA-specific CD4 T cell line prevented colitis. Thus, an expansion of CD4 T cells monospecific to OVA, an antigen non-cross-reactive to colonic tissue, can mediate both induction and inhibition of the colitis which was associated with hyperplasia of lymph follicles.

Introduction

The intestinal immune system, which contains a considerable proportion of the total body lymphocytes, is in close proximity to the myriads of antigens derived from food and indigenous microorganisms in the intestine. Normally, cell-mediated immune reaction by the host to these luminal antigens is tolerated in the gut. Indeed, p.o. exposure to an antigen can lead to a subsequent state of systemic hyporeactivity to the fed antigen, suggesting the presence of mechanisms actively suppressing immune responses to the antigens in the gut. Because of the large variety of antigens and the large number of lymphoid cells in the intestine, perturbation in the systemic immune network could result in intestinal immune dysfunction.

Indeed, recent years have seen the development of various animal models of colitis (1,2), which are intestinal manifestations of the systemic immune dysregulation either induced or spontaneously acquired in immunodeficient mice. These colitis models can be grouped into four categories by the method inducing perturbation of homeostasis in the mucosal immune system: (i) alterations in the T cell subsets either by abnormal selection at the thymus (3), SCID adoptive transfer of CD45 RB⁺ cells (4,5) or induced mutation in TCR-α chain genes (6), (ii) cytokine gene knockouts or transgenic mice (7–9), (iii) mice lacking signaling peptide (Gα2) (10) and (iv) direct instillation of haptenic chemical reagents to the colonic...
mucosa (11–14). The first three types of colitis models share the fact that the mice have systemic immunodeficiencies, and the colitis is spontaneous in onset and chronic in clinical course. In contrast, the last category of colitis is induced in immunocompetent mice and assumes a more acute course; however, even in this case, pre-existing genetic factors exist. Thus, colitis is only induced in certain strains of mice. One feature common to most of the above animal models is that colitis is either attenuated or does not occur at all by restricting bacterial colonization (15–18). Thus the microflora in the intestine appeared to play a pivotal role in the pathogenesis of experimental colitis (19). Recent studies on a SCID transfer model (20) and TCR α chain knockout (21) model indicate oligoclonal expansion of CD4 T cells presumably driven by microbial antigens. A study on colitis in C3H/HeJBlr mice showed induction of colitis in SCID hosts upon transfer of pauciclonal CD4 T cell lines specific to the colonic bacterial antigens which have not yet been identified (22).

In order to study directly the relevance of clonal expansion of bacteria-specific CD4 T cells to the induction of colitis, we have created *Escherichia coli* producing model antigens and a cell transfer model that allows us to test the colitogenic potency of the transferred CD4 T cells specific to the antigen produced by those *E. coli*. We show here that both development and suppression of a colitis can be produced by introduction of CD4 T cells specific to bacteria-associated ovalbumin (OVA), an antigen non-cross-reactive to colonic tissue.

**Methods**

**Animals**

BALB/c mice and C.B-17 scid/scid (SCID) mice (8–12 weeks old) were purchased from Shizuoka Laboratory Animal Center (Hamamatsu, Japan) and Clea Japan (Osaka, Japan) respectively. Two strains of BALB/c mice expressing a transgenic TCR were used in this study: Rag-2 knockout mice crossed to OVA-23-3 (23) (Rag-2/OVA-TCR-Tg) and DO11.10 (24), fl mice which were kindly provided by Dr Kenneth M. Murphy (Washington University). Both transgenic TCR recognize the identical 323–339 peptide fragments of OVA in the context of I-A^d^. Mice were housed under specific pathogen-free conditions at the Animal Facilities of Kyoto University, Department of Medicine. All animal experimentation was performed in accordance with institutional guidelines and ethical permission for this study was granted by the Review Board of Kyoto University.

**Generation of *E. coli* expressing OVA**

PEZZ 18 vector was purchased from Amersham Pharmacia Biotech (Little Chalfont, UK). This plasmid has a promoter, a signal sequence, a region coding two Z domains of Protein A, a multi-cloning site, cDNA of β-galactosidase (LacZ) and an ampicillin resistance gene. A cDNA fragment containing the entire coding region of the chicken OVA gene was created as previously described (25) and subcloned into a multi-cloning site. *E. coli* (strain JM109; Toyobo, Osaka, Japan) transformed with these OVA and LacZ expression constructs, designated as ECOVA and ECLacZ respectively, constitutively secreted chimeric molecules fused to a domain of Protein A binding to IgG at its N-terminus. Recombinant OVA and LacZ secreted into culture supernatant were concentrated with Centricon (Millipore, Bedford, MA) and detected by Western blot analysis using alkaline phosphatase-labeled goat IgG (Life Technologies, Gaithersburg, MD) which binds to the Protein A moiety of fusion proteins.

**Determination of anti-OVA antibody**

In order to determine the titer of anti-OVA antibodies, sera and fresh feces were collected from non-transgenic BALB/c mice, which were inoculated with 1×10^8^ ECOVA and ECLacZ per rectum once a week for 1 month after initial treatment of 100 µl of 50% ethanol. Serum IgG and secretory IgA specific to OVA were measured by standard ELISA.

**T cell proliferation assay**

CD4 T cells were positively selected by magnetic sorting utilizing MACS immunobeads (Miltenyi Biotech, Bergish Gladbach, Germany). Purity of CD4 T cells was usually >95%, when tested by flow cytometry. For T cell proliferation assay, splenic CD4 T cells (1×10^6/ml) purified from BALB/c mice inoculated with ECOVA or ECLacZ were stimulated with OVA (100 µg/ml; Sigma, St Louis, MO) presented by irradiated splenocytes (1×10^7/ml) of non-transgenic BALB/c mice. The cells were pulsed with [3H]thymidine at day 3 for the last 12 h of the culture and measured by a Topcount microplate scintillation counter 9912V (Packard, Meriden, CT).

**Protocol for the induction of colitis**

For the induction of colitis, BALB/c and SCID mice were injected i.v. with 1×10^7^ of CD4^+^ splenocytes prepared from Rag-2^−/−^/OVA-TCR-Tg mice. For staining of OVA-specific CD4 T cells, 1×10^7^ CD4 splenocytes from DO11.10 mice were used as donor cells. Challenge of recipient mice with *E. coli* and assessment of colonization were done as described previously (26). In brief, for the first 3 days after cell transfer, mice were given streptomycin (Meiji Seika, Osaka, Japan) in drinking water (5.0 g/l). After 24 h of fasting, an elastic catheter of 3.5 Fr (Fuchigami, Tokyo, Japan) was carefully inserted as far into the colon as 4.5 cm from the anus and 100 µl of 50% ethanol was slowly instilled into the lumen of the colon. This ethanol treatment was done only at day 1. Six hours after the ethanol treatment, all mice were inoculated with 1×10^8^ c.f.u. of ECOVA or ECLacZ in 100 µl of PBS or 100 µl of OVA solution in PBS (100 mg/ml) per rectum everyday during the observation period. During the inoculation and subsequent observation period, all mice were given drinking water containing ampicillin (1.0 g/l; Nakarai Tesque, Kyoto, Japan) ad libitum and housed in isolation cages with filter hoods during the whole period of experiment. For the assessment of the colonization of the colon by *E. coli*, fresh feces were obtained at various time points and suspended in PBS (0.1 mg/ml). An aliquot of this suspension was spread onto an LB agar plate containing ampicillin. A positive culture was confirmed by isolation of the predicted plasmid from the colony.

**Evaluation of histopathology**

Hematoxylin & eosin-stained paraffin sections were evaluated by two pathologists blind to the treatment of the mice.
A colitis induced by OVA-specific T cells

Fig. 1. Secretion and immunogenicity of OVA expressed in E. coli. The culture supernatant of ECLacZ and ECOVA were analyzed by Western blot. OVA and LacZ secreted in the media concentrated 10-fold were detected by alkaline phosphatase-labeled rat IgG (a). OVA-specific proliferation of splenic CD4 T cells (b) in non-transgenic BALB/c mice inoculated with ECOVA or ECLacZ per rectum once a week for 1 month. The mean values and SD of each group (n = 5) are shown (*P < 0.01).

Fig. 2. The mean body weight of BALB/c mice inoculated with ECOVA and ECLacZ. BALB/c mice were injected i.v. with CD4+ splenocytes prepared from Rag-2+/-/OVA-TCR-Tg mice. Four days after cell transfer, mice were treated with 50% ethanol and inoculated with ECLacZ or ECOVA, or OVA solution per rectum everyday. The mean percentages and SD of each group (n = 6) at indicated time points are shown (*P < 0.01). Results shown are representative out of three independent experiments.

Histological features of inflammation were graded from 0–4, as described previously (12).

Immunohistochemistry of T cells and OVA-specific T cells
Samples were put into OCT compound and 6-μm cryosections were produced by standard procedures. Pan-T cells or DO11.10 clonotype-positive cells (OVA-specific T cells) were stained with biotinylated anti-CD3 (clone 145-2C11; PharMingen, San Diego, CA) or with sequential KJ1-26–FITC (Caltag, Burlingame, CA) and then biotinylated anti-FITC (Molecular Probes, Eugene, OR) respectively. Next, these sections were incubated with avidin–biotin complex (Vector, Burlingame, CA) for 30 min and then incubated with DAB solution (Vector). Finally, nuclei were stained by Methyl Green (Dako, Carpinteria, CA).

Isolation of lamina propria (LP) CD4 lymphocytes and cytokine assay
Colonic LP lymphocytes were isolated by the method described previously (27). LP CD4 lymphocytes were positively selected by an immunobeads method as described above. The isolated lymphocytes were >90% viable by Trypan blue dye exclusion. The ability of cytokine production in each LP CD4 lymphocytes (1×10⁶/ml) purified from colonic tissue was assessed by stimulating an aliquot of T cells with OVA peptide (1 μg/ml) presented by irradiated splenic antigen-presenting cells (APC) (1×10⁶/ml) in the microwell for 48 h. The amounts of IFN-γ, IL-4 and IL-10 in culture supernatant were measured by cytokine-specific ELISA (IFN-γ and IL-10; PharMingen, San Diego, CA and IL-4; Endogen, Woburn, MA).

Generation of CD4 T cell lines producing IL-10
Antigen-specific CD4 T cells producing IL-10 were generated by the method described previously (28) with minor modifications. CD4 T cells purified from Rag-2+/-/OVA-TCR-Tg mice and from BALB/c mice immunized with human serum albumin (HSA; Sigma) were stimulated with 1 μg/ml OVA peptide or with 10 μg/ml of HSA respectively in the presence of irradiated splenic APC (1×10⁷/ml), 300 ng/ml rIL-10 and 20 U/ml rIL-2 (Peprotech, Rocky Hill, NJ). The cells were re-stimulated with OVA peptide under the same conditions once a week, repeatedly, and used after 5 times re-stimulation. To determine the efficacy for prevention of colitis, CD4 T cell lines (2.5×10⁵/mouse) thus generated were transferred to recipient mice together with 1×10⁷ CD4+ splenocytes purified from Rag-2+/-/OVA-TCR-Tg mice before challenge with the E. coli. In this co-transfer study, recipient mice were treated with an i.p injection of anti-IL-10 antibody (1 mg/mouse) (JESS-A5, kindly provided by Dr Hiroshi Ishida) or control rat IgG every other day for 1 week. The ability of cytokine production by these T cell lines was assessed by stimulating aliquots of T cells with an immobilized anti-CD3 antibody (2C11; PharMingen) in the microwell for 18 h. The amounts of IFN-γ, IL-4, IL-10 and transforming growth factor (TGF)-β in culture supernatant were measured by cytokine-specific ELISA (Promega, Madison, WI) to monitor the appearance of an appropriate line. To measure the amounts of TGF-β, RPMI medium without FCS was used.

Statistical analysis
The results were expressed as the mean ± SD. Statistical analysis of antibody titer, cell proliferation assay and body weight change were assessed by Student’s t-test. Comparison
A colitis induced by OVA-specific T cells

Results

Creation of E. coli expressing model antigens

A previous study reported that the bacterial antigens associated with a colitis have the properties of protein and can be presented in a class II-dependent manner (29) Considering this and planning to use T cells bearing a TCR with defined specificity, we created E. coli producing model antigens, OVA and LacZ. These E. coli, designated as ECOVA and ECLacZ, produced the respective protein in the culture medium (Fig. 1a). The immunogenicity in vivo of these proteins was tested by inoculation of ECOVA and ECLacZ per rectum to BALB/c mice. After an initial ethanol treatment followed by inoculation of ECOVA or ECLacZ once a week for 4 weeks, we measured titers of antibodies in sera and proliferative response by splenic CD4 T cells specific to OVA. Only the mice administered with ECOVA showed an increase in the titer of anti-OVA IgG [ECOVA 0.22 ± 0.08 (OD) versus ECLacZ 0.04 ± 0.02, P < 0.01] and IgA [ECOVA 0.21 ± 0.06 (OD) versus ECLacZ 0.03 ± 0.01, P < 0.01]. In addition, as shown in Fig. 1(b), splenic CD4 T cells prepared from the mice inoculated with ECOVA showed significant proliferation upon re-stimulation with OVA, whereas those from the mice with ECLacZ did not. OVA purified from the culture supernatant of ECOVA was recognized by CD4 T cells obtained from Rag-2^−/−/OVA-TCR-Tg mice (data not shown). Thus, ECOVA elicited an anti-OVA response in the mice challenged via the rectal route.

ECOVA induces wasting disease in mice transferred with CD4 T cells specific to OVA

In the next series of studies, we determined whether administration of ECOVA to the host who has OVA-specific T cells would lead to colonic inflammation. To this end, we adoptively transferred CD4^+^ splenocytes prepared from Rag-2^−/−/OVA-TCR-Tg mice into BALB/c mice and, after 4 days, inoculated with ECOVA or ECLacZ per rectum every day. The mice inoculated with ECOVA showed ruffling of body hair, diarrhea, rectal prolapse and significant body weight loss that peaked at day 3 and gradually restored in 24 days (Fig. 2). However, the mice with ECLacZ, or mice repeatedly instilled with the OVA solution, showed no such changes. The density of ECOVA recovered from feces was no less than that of ECLacZ (data not shown). The grade of weight loss correlated with the dose of CD4^+^ T cells transferred (data not shown). In fact, at a cell dose of 1×10^5^ CD4^+^ splenocytes we did not see any significant weight loss. However, at a cell dose of 1×10^7^ CD4^+^ splenocytes we did see a significant body weight loss that peaked at day 3 and gradually restored the original body weight in 24 days. Thus, transfer of OVA-specific CD4 T cells can lead to a wasting disease in mice inoculated with ECOVA.

Colonic pathology of BALB/c mice inoculated with ECOVA

With clinical findings indicative of colitis in mice with ECOVA and OVA-specific CD4 T cells, we next studied macro- and
A colitis induced by OVA-specific T cells

Fig. 4. Photomicrographs of ECOVA-induced colitis in SCID recipients (a–c and e–g) at day 4. The SCID recipients with CD4+ splenocytes of Rag-2−/−OVA-TCR-Tg mice were inoculated with ECLacZ (a, ×100; b, ×200; c, ×100) or ECOVA (e, ×100; f, ×200; g, ×100). Focal infiltration by mononuclear cells, destruction of crypt architecture and loss of goblet cells are seen in the mice colonized with ECOVA. Immunostaining of T cells with anti-CD3 antibody (d and h). T cells were detected in the colonic lymph follicles (arrowhead) and LP (arrow) of the colon in mice inoculated with ECOVA.

ECOVA-induced colitis in SCID mice

Next, to characterize the cells causing ECOVA-induced colitis, we adoptively transferred CD4+ splenocytes prepared from Rag-2−/−OVA-TCR-Tg mice into SCID mice and inoculated with ECOVA or ECLacZ per rectum every day. Figure 4(e–g) shows colitis in the colon of the SCID mice inoculated with ECOVA, whereas no colitis in SCID mice with ECLacZ (Fig. 4a–c) was seen. Clinical findings and histological features of the colitis in SCID mice were similar to those in BALB/c mice. In terms of immunohistochemistry, remarkable infiltration by CD3+ cells was detected in the colonic patches and LP of SCID mice as was the case with BALB/c mice (Fig. 4h). Thus, antigen-specific T cells are sufficient, and B cells and antigen-non-specific T cells were not required for the development of ECOVA-induced colitis.

Cytokine production by LP lymphocytes of BALB/c mice inoculated with ECOVA or ECLacZ

To determine the nature of immune reaction in the diseased colon of the mice with ECOVA inoculation, we measured cytokines produced by LP CD4 T lymphocytes (Fig. 5). We purified colonic LP CD4 T cells at day 2 and 5 from BALB/c mice inoculated with E. coli and re-stimulated these cells with APC pulsed with OVA peptide and measured IFN-γ, IL-4 and IL-10 secreted into the culture medium. At day 2, LP CD4 T cells in mice inoculated with ECOVA produced large amounts of IFN-γ. No detectable IL-4 and IL-10 were produced at this time point. However, at day 5, IL-4 and IL-10 production by LP CD4 T cells in mice inoculated with ECOVA produced large amounts of IFN-γ. No detectable IL-4 and IL-10 were produced at this time point. However, at day 5, IL-4 and IL-10 production by LP CD4 T cells in these mice increased, which was associated with the decrease of IFN-γ production. On the contrary, in mice inoculated with ECLacZ, no detectable amounts of IL-4 and IL-10, and very small amounts of IFN-γ were secreted by colonic LP CD4 T cells at these time points. Thus, it is suggested that, during the course of colitis, OVA-specific CD4 T cells secreting Th1-type lymphokines emerge first in the colon which is subsequently infiltrated by OVA-specific CD4 T cells producing IL-4 and IL-10 at the later time point.
An inhibitory effect of OVA-specific CD4 T cells producing IL-10 in ECOVA-induced colitis

Since OVA-specific T cells producing IL-10 prevent colitis in the SCID transfer model in which CD45RB<sup>hi</sup> CD4 T cells are injected (28), we tested whether the emergence of CD4 T cells producing IL-10 was associated with the healing of colitis in our model. To this end, we generated OVA-specific CD4 T cell lines producing IL-10. Lymphokines produced in the culture supernatants were determined by ELISA. Mean values and SD are shown (*<i>P</i> < 0.01).

![Fig. 5. Lymphokine production by LP CD4 T lymphocytes at day 2 and 5. LP CD4 lymphocytes were extracted from the colon of the BALB/c mice transferred with CD4<sup>+</sup> splenocytes of Rag-2<sup>–/–</sup>/OVA-TCR-Tg mice and inoculated with ECOVA or ECLacZ. The CD4 T cells were stimulated with APC pulsed with an OVA peptide for 48 h. After the stimulation, IFN-γ (a), IL-4 (b) and IL-10 (c) produced into the culture supernatants were measured by ELISA. Mean values and SD are shown (*<i>P</i> < 0.01).](image)

Table 1. Histological evaluation of colitis in the BALB/c recipient mice with transferred CD4 T cells obtained from Rag-2<sup>–/–</sup>/OVA-TCR-Tg T cells and colonized with <i>E. coli</i> at day 4

<table>
<thead>
<tr>
<th>Histological score</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECLacZ</td>
<td>5</td>
<td>4</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ECOVA</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>

Colonic specimens were taken 3 days after administration of ECLacZ or ECOVA and the magnitude of inflammatory changes in the colonic tissue was analyzed on hematoxylin & eosin-stained cross-sections. Data were pooled from two independent experiments.

Table 2. Cytokines produced by OVA-specific CD4 T cell lines stimulated with an immobilized anti-CD3 antibody for 48 h

<table>
<thead>
<tr>
<th>IFN-γ (ng/ml)</th>
<th>IL-4 (pg/ml)</th>
<th>IL-10 (pg/ml)</th>
<th>TGF-β (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2A5</td>
<td>15.3 ± 2.2</td>
<td>58.3 ± 4.5</td>
<td>1250 ± 190</td>
</tr>
<tr>
<td>3D6</td>
<td>20.7 ± 3.5</td>
<td>125 ± 10.5</td>
<td>815 ± 93</td>
</tr>
<tr>
<td>4E1</td>
<td>24 ± 3.7</td>
<td>65.3 ± 7.8</td>
<td>320 ± 34</td>
</tr>
</tbody>
</table>

CD4 T cells purified from Rag-2<sup>–/–</sup>/OVA-TCR-Tg mice were stimulated once a week with OVA peptide (1 μg/ml) in the presence of IL-10 (300 ng/ml) and IL-2 (20 U/ml). After 5 times stimulation, cells were cloned by limiting dilution in the presence of IL-2. Cytokines were determined by ELISA of culture supernatant. Results indicate mean ± SD representing pooled data from three representative experiments.

In histopathology, the destruction of the crypt architecture and the inflammatory infiltrates were less in the mice with co-transfer, the effect of which was also abolished by anti-IL10 antibody treatment (Fig. 6c and d). Thus, altogether, these results indicate that OVA-specific T cells producing IL-10 prevent colitis in our model.

Discussion

The results of this study provide clear evidence that OVA, an antigen non-cross-reactive to colonic tissue, can induce colitis in the genetically susceptible host by being delivered by intestinal microflora. This model also suggests that clones of colitogenic T cells expand in the colonic lymph follicles and that the expansion of monospecific CD4 T cells can lead to a colitis.

Several features of the colitis described here are worthy of emphasis. First, the conditions for the development of colitis in this model are not dissimilar to those occurring in the SCID transfer model in which CD45RB<sup>hi</sup> CD4<sup>+</sup> T cells are injected and then react with normal flora in the absence of counter-regulatory T cells, or indeed in the IL-10<sup>–/–</sup> and IL-2<sup>–/–</sup> mice which develop colitis under conventional breeding conditions. In fact, CD4 T cells transferred in our model are from Rag-2<sup>–/–</sup> OVA-TCR-Tg mice and are virtually all CD45RB<sup>hi</sup>. In addition,
Fig. 6. Prevention of colitis by co-transfer of OVA-specific CD4 T cells producing IL-10. Changes of the body weight in SCID (a) or BALB/c (b) recipients which were co-transferred with 1\times10^7 of CD4^+ splenocytes of Rag-2^−/−/OVA-TCR-Tg and 2.5\times10^5 OVA-specific CD4 T cell lines producing various amount of IL-10 (2A5, □, ■, 3D6, ○, ●, 4E1, △, ▲) followed by inoculation with ECOVA and i.p. injection of anti-IL10 (solid symbols) or control antibody (open symbols). Percentages of mean body weight and SD in each group (n = 5) were determined at the indicated time points. Results shown are representative out of three independent experiments. The co-transferred CD4 T cell line producing IL-10 prevented weight loss in SCID (a) and BALB/c (b) recipients for the whole observation period; the effect in the latter mice was neutralized by anti-IL-10 antibody. Representative histopathologies of the mice transferred with 2A5 with control antibody (c) and anti-IL-10 antibody (d) are shown.

some reports indicate that T cells reacting against a bacterial flora antigen are present and responsible for the colitis, so that colitis does not develop or is attenuated under germ-free conditions in these mice (18,30). Although lack of colitis in germ-free mice may be partly due to the fact that those axenic mice do not have sufficient intestinal T cells to induce colitis, a recent experiment by Sellon et al. showed that limitation of bacterial colonization in IL-10 knockout mice can also prevent colitis (15).

Second, the histopathology of the induced colitis consisted of non-diffuse type infiltration by mononuclear cells in the LP and submucosa. This feature of focal involvement is similar to that seen in a colitis model in SCID hosts transferred with T cell lines which were established from C3H/HeJBir mice and reactive to colonic bacterial antigens (22). Destruction of crypt architecture, focal lymphoid aggregation, loss of goblet cells and occasional crypt abscesses were seen in our model. Importantly, there was no involvement of the muscle layers and no granuloma formation. This pathological picture is more akin to ulcerative colitis than Crohn’s disease in humans. However, as discussed below, the inflammation in the model described here occurs along with a cytokine elevation indicative of a Th1 T cell response, which is different from that of ulcerative colitis, since the latter is usually not associated with a Th1 T cell cytokine profile.

Third, as alluded to above, studies of cytokine profiles indicated that, in BALB/c mice repopulated with Rag-2^−/−/OVA-TCR-Tg T cells (BALB/c origin), inoculation of ECOVA led to a typical Th1 T cell cytokine response marked by elevations of IFN-γ and undetectable levels of IL-4 and IL-10. This cytokine response pattern is somewhat surprising given the tendency of BALB/c mice to develop predominantly Th2 cytokine responses following infectious challenge or intracolonic haptenic challenge (31). Evidently, the colitis-inducing stimulus delivered to these mice results in a strong Th1 T cell response at an early time point of the colitis, which was overcome by their inherent Th2 driving mechanisms at a later time point.

Fourth, colitis described in this model is short lived; mice restored their original body weight in 24 days. The remission of the colitis was associated with a decrease of IFN-γ, and an increase of IL-4 and IL-10 secretion by LP CD4 T cells specific to OVA. This can be a feedback counter-regulatory cytokine response by the CD4 T cells specific to the identical
OVA peptide. The shift of cytokine profile in LP CD4 T cells in response to OVA could be explained by the switch of regional APC—from T\(_{\text{H}}\)1-promoting dendritic cells, which initiate a regional immune response and potentiate high IFN-\(\gamma\) secretion capacity in effector CD4 T cells, to T\(_{\text{H}}\)2-promoting dendritic cells (32,33). In fact, we saw co-localization of CD11c\(^+\) cells to KJ1-26\(^+\) cell in the colonic tissue of the mice challenged with ECOVA (data not shown). Thus, it has to be determined in the future whether a functional shift of APC associates with the shift of the cytokine profile of OVA-specific T cells in our model. Alternatively, OVA produced by the ECOVA inoculated every day might have preferentially deleted the OVA-specific T\(_{\text{H}}\)1-type cells, which are more prone to cell death by apoptosis than the CD4 T cells producing IL-4 (34–36). Thus, at the time point where ECOVA is still present, emergence of T\(_{\text{H}}\)2- and/or T\(_{\text{H}}\)1-like cells in the LP could have been a result of the apoptosis of T\(_{\text{H}}\)1-like cells present in the cell population transferred. To formally prove active suppression of colitis by the former subsets of CD4 T cells, we transferred OVA-specific T\(_{\text{H}}\)1-like cells prior to ECOVA inoculation. Indeed, we saw prevention of colonic inflammation and weight loss in the mice co-transferred with T\(_{\text{H}}\)1-like cells. This protective effect appeared to correlate with the potency of IL-10 secretion by transferred CD4 T cell lines and, more importantly, the protective effect was abolished by anti-IL10 antibody treatment of the recipient mice. Furthermore, we did not see significant protection by co-transfer of IL-10-producing CD4 T cell lines specific to HSA (data not shown). This may indicate that TCR-mediated cell activation is required for the T\(_{\text{H}}\)1-like cells to suppress colitis, which is compatible with the previous report (28). Although the level of IL-10 increases in the tissue at the recovery stage and transfer of in vitro generated T\(_{\text{H}}\)1-like cells suppresses colitis, it still remains an open question whether T\(_{\text{H}}\)1 cells as defined (28) would emerge in vivo and secrete IL-10 at the recovering stage. In addition, we do not preclude the possibility that OVA non-specific T cells and/or B cells may also play roles in regulation of the colitis, as indicated in the recent report that B cells play a suppressive role in the development of spontaneous colitis in TCR \(\alpha\)-knockout mice (37). This question on the role played by cells other than OVA-specific effector CD4 T cells warrants further study utilizing ECOVA in the SCID transfer model.

As for the mechanism of the colitis induction in ECOVA-colonized mice, we would postulate that an antigen (in this case OVA) delivered by antigen-secreting \textit{E. coli} is captured by mucosal dendritic cells and transported to a regional T cell area of a lymphoid follicle and expands OVA-specific CD4 T cells. Indeed, we spotted CD11c\(^+\) cells in very close proximity to KJ1-26\(^+\) cells in the follicles (data not shown). In SCID mice transferred with OVA-specific CD4 T cells and inoculated with ECOVA, we saw a colitis with microscopic features of focal infiltration by lymphocytes and enlargement of colonic lymph follicles, suggesting an expansion of OVA-specific CD4 T cells in these follicles. Thus, follicular hyperplasia and focal infiltration by mononuclear cells are conspicuous features of ECOVA-induced colitis of the mice with OVA-specific CD4 T cells. This may relate to recent reports showing that removal of the appendix, an aggregate of lymph follicles, prevents colitis in both humans and mice (38,39). This may also explain the reason that we did not see significant colitis in the mice instilled with OVA solution under continuous treatment of antibiotics before and during instillation. Since bacteria-derived inflammatory signals are virtually absent, cell migration, antigen processing and presentation by regional dendritic cells are presumably very limited in those mice. It is also possible that repeated instillation of soluble OVA may lead to deletion or anergy of the OVA-specific T cells. Alternatively, soluble OVA might not stay in the colon long enough to elicit an inflammatory response. It would be worthwhile to test whether soluble OVA or OVA peptide could elicit colitis in the mice in which bacteria-derived factors are provided.

Since intestinal microbial antigen(s) can drive clonal expansion of T\(_{\text{H}}\)2-type CD4 T cells and induce colitis in such models as TCR \(\alpha\)-deficient mice (40) and 2,4,6-trinitrobenzene sulfonic acid-treated BALB/c mice (31), it now appears that polarized expansion of bacteria-specific CD4 T cells, either T\(_{\text{H}}\)1 or T\(_{\text{H}}\)2, can lead to colitis. Thus we postulate that unbalanced colonic expansion of OVA-specific CD4 T cells brought by transfer of monospecific CD4 T cells and continuous delivery of OVA by ECOVA are the mechanisms responsible for colitis in our model. Therefore, it will provide us with an important insight into the pathogenesis of the colitis if we administer IL-4 and anti-IFN-\(\gamma\) antibody to IL-12-deficient recipient mice and get T\(_{\text{H}}\)2-type colitis in this model. It still remains to be answered why expansion of T\(_{\text{H}}\)1 cells proceeds and predominates over T\(_{\text{H}}\)2 cells in our model, when T\(_{\text{H}}\)2 cells could preferentially expand in other models.

The ability to cause colitis with a defined bacterium secreting an inciting antigen provides a unique opportunity to study the conditions necessary for the induction of colitis and its ability to sustain itself. On this basis, the model reported here as well as a variation of this model in which the antigen is delivered by luminal microorganisms will be of considerable importance.

**Acknowledgements**

We thank Dr Warren Strober (NIH) for his critical reading of the manuscript, Dr Yasuhiro Nagahama (Kyoto University) for his statistical analysis and Miss Naoko Sakanashi for her secretarial assistance. This work was supported in part by grants from the Ministry of Education, Science and Culture, Japan (Y. W.), the Japan Society for the Promotion of Science (JSPS) (Y. W. and M. Y.), the Dr Shimizu Foundation for the Promotion of Immunology Research Grant (Y. W.), and the Yakult Bioscience Foundation (Y. W.).

**Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>APC</td>
<td>antigen-presenting cell</td>
</tr>
<tr>
<td>ECLacZ</td>
<td>\textit{Escherichia coli} expressing LacZ</td>
</tr>
<tr>
<td>ECOVA</td>
<td>\textit{Escherichia coli} expressing ovalbumin</td>
</tr>
<tr>
<td>HSA</td>
<td>human serum antigen</td>
</tr>
<tr>
<td>IBD</td>
<td>inflammatory bowel disease</td>
</tr>
<tr>
<td>LacZ</td>
<td>(\beta)-galactosidase</td>
</tr>
<tr>
<td>LP</td>
<td>lamina propria</td>
</tr>
<tr>
<td>OVA</td>
<td>ovalbumin</td>
</tr>
</tbody>
</table>
References


A colitis induced by OVA-specific T cells

Stat6 is probably not required for the effect of IL-4. J. Exp. Med. 186:325.


