Acta Medica et Biologica
Vol. 50, No.1, 25-32, 2002

The Effect and Mechanism of 5-Aminosalicyclic Acid Enema Treatment on Experimental Colitis Induced by a Murine Retrovirus

Tsuneo AIBA[1], Kenji SUZUKI[1], Yasuyuki BABA[1], Hidehisa SURIKI[1], Shunya SASAKI[1], Shiro WATANABE[1], Yusuke KAWAUCHI[1], Hiroshi KAWACHI[2], Fujio SHIMIZU[2] and Hitoshi ASAKURA[1]

[1]Division of Gastroenterology and Hepatology, Department of Cellular Function, and [2]Division of Cell Biology, Department of Nephrology, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan

Received December 25 2001; accepted January 23 2002

Summary. **LP-BM5 murine leukemia virus is known to induce murine acquired immunodeficiency syndrome (MAIDS). We have shown that adoptively transferred spleen cells of MAIDS mice can induce inflammatory bowel disease (IBD)-like colitis, which we termed "MAIDS colitis", in nude mice. Although 5-aminosalicylic acid (5-ASA, mesalazine) is clinically used for successful treatment for IBD, its precise mechanism remains largely unknown. To reveal the effect and mechanism of 5-ASA treatment for our colitis model, four-week-old C57BL/6 (B6) mice were inoculated intraperitoneally with LP-BM5; 8 weeks after infection, lymphnode cells from the mice were transferred into B6 nude mice intraperitoneally to induce MAIDS colitis. The mice were grouped as follows: Group 1, MAIDS colitis mice; Group 2, MAIDS colitis mice treated with only sodium carboxymethylcellulose (CMC) enema; Group 3, MAIDS colitis mice treated with 5-ASA and CMC by enema every day after cell transfer; Group 4, Control B6 nude mice treated with CMC enema. Four to six weeks after cell transfer, 4 mice of each group were killed and the colon was removed for further analyses. The expression of interferon-γ and interleukin-10 protein was detected by a double color-staining immunofluorescence technique. MAIDS colitis mice showed diarrhea and a bloody stool. Ulcerative colitis (UC)-like colitis lesions were observed histologically, as evidenced by cellular infiltration in the mucosal and submucosal layers crypt abscess and erosions of the colon. In contrast, the number of infiltrating cells was decreased in the colon of MAIDS colitis mice treated with 5-ASA enema. Especially, Mac-1$^+$ cells (mainly macrophages) and CD4 positive cells that were positive for IFN-γ or IL-10 were reduced in the colon of MAIDS colitis mice undergoing a 5-ASA enema. Colitis did not develop in the control mice. Thus, 5-ASA enema is effective for MAIDS colitis as well as human IBD. One of the therapeutic effects of 5-ASA on IBD might be the suppression of macrophages and CD4$^+$ T lymphocytes in the colon.**

Key words—5-aminosalicyclic acid, inflammatory bowel disease, ulcerative colitis, murine AIDS, LP-BM5 murine leukemia virus.

INTRODUCTION

Inflammatory bowel diseases (IBD), which are mainly composed of ulcerative colitis (UC) and Crohn's disease (CD), are chronic inflammatory disorders of the gut with an unknown etiology. To reveal the etiopathogenesis and pathophysiology of IBD, many animal models have been developed, including inter leukin (IL)-2-deficient mice[1], TGF-β1-deficient mice[2,3], and SCID mice that have been transferred with CD45RB^{high} T cells[4]. Analyses of these mice have strongly suggested that a strong polarization of mucosal T cell responses along either Th1 or Th2 pathway can result in IBD, and that among the many cytokines, IFN-γ and IL-10 play a key role in the pathogenesis of IBD. These models have been also used to develop a new therapeutic approach to IBD. For example, anti-inflammatory cytokine, IL-10 has been shown to play a protective role in IBD by the generation of IL-10 deficient mice that spontaneously develop IBD[5]. With these experimental backgrounds,

Correspondence: Kenji Suzuki, M.D., Ph.D., The Third Department of Internal Medicine, Niigata University School of Medicine, 1-757 Asahimachi-dori, Niigata 951-8122, Japan.

IL-10 has been used for patients with CD and UC, and good clinical outcomes have been reported[6]. Thus, animal models for IBD are powerful tools for studying IBD.

The LP-BM5 murine leukemia virus (MuLV) is a retrovirus that is known to induce murine acquired immunodeficiency syndrome (MAIDS)[7]. We have previously reported that systemic exocrinopathy resembling Sjögren's syndrome (SjS) was induced in the virus-infected mice[8]. In addition, nude mice inoculated with lymph node cells from mice with MAIDS developed IBD-like colitis, which we termed 'MAIDS colitis', as well as SjS-like exocrinopathy[9]. Histopathologically, MAIDS colitis mimics UC with an infiltration of inflammatory cells into the mucosal and submucosal layers of the colon, mucosal erosion, and hyperplasia of epithelial cells. In the colon of MAIDS colitis, Th2 type $CD4^+$T cells predominated over Th1 type $CD4^+$ T cells, and IFN-γ or IL-10 positive macrophages were also detected, especially beneath the eroded epithelial lesions of the colon[10]. In our colitis model, Mac-1^+ macrophages that produce both IFN-γ and IL-10 might play a crucial role in the pathogenesis of colitis in combination with $CD4^+$ T cells[10].

Salazosulphapyridne (SASP) is widely used as a mainstay for the treatment of inflammatory bowel disease. The compound is characterized by the azo linkage of sulphapyridine (SP) and mesalazine (5-aminosalicylic acid; 5-ASA)[11]. 5-ASA is thought to be the active component of SASP, while SP is considered to be responsible for adverse reactions such as malaise, nausea, abdominal discomfort, and headache[11,12]. Mesalazine microgranules (Pentasa^R) is a 5-ASA preparation in the form of microgranules coated with ethylcellulose[12]. This form ensures the controlled release of 5-ASA from the small intestine right through to the rectum. Pentasa has recently been introduced and approved as a treatment for UC in many countries, and has been reported to be as effective as sulphasalazine[13,14]. Meta-analysis by Marshall and Irvine suggests that rectal 5-ASA is as efficacious as rectal corticosteroids for alleviating the disease and is better than rectal corticosteroids for inducing remission[15]. However, the mechanism of action of the drug has not been fully clarified.

In this study we analyzed the effect of 5-ASA enema in the colon of MAIDS colitis model mice using the double-color immunofluorescence (IF) technique in order to elucidate the mechanism of the action of this drug.

MATERIALS AND METHODS

Animals

Female C57BL/6 (B6) mice were purchased from Charles River Japan (Atsugi, Kanagawa, Japan). The B6 nude mice were provided by Dr. Norimitsu Sato of the Animal Center, Niigata University School of Medicine. All mice were maintained at the same animal center under specific pathogen-free conditions. All animal experiments were performed according to the "Guide for Animal Experiments" of the Niigata University School of Medicine.

Virus

LP-BM5 MuLV was prepared from the supernatant of cloned G6 cells infected with the retrovirus. A twenty-four-hour culture supernatant of G6 cells contained approximately 5×10^4 plaque-forming units per milliliter of ecotropic virus, as determined by XC plaque assay. Aliquotes containing the virus were stored at -80℃ until use. For the infection, 4-week-old B6 mice were inoculated intraperitoneally with 0.3 ml of the stock solution of LP-BM5 MuLV.

Induction of MAIDS and MAIDS colitis

Four-week-old B6 female mice were injected intraperitoneally with 0.3 ml of LP-BM5 MuLV virus stock solution. Induction of MAIDS was confirmed when the mice developed splenomegaly and generalized lymphadenopathy. Eight weeks after the viral inoculation, mice with MAIDS were killed by cervical dislocation under ether anesthesia, and their lymph nodes were collected. The lymph nodes were pressed and passed through a steel mesh, and the cell suspension was transferred intravenously to 10- to 13- week-old female B6 nude mice at a dose of 5×10^7 lymph node cells per head. Symptoms of colitis such as diarrhea and anal bleeding were observed 4 weeks after cell transfer, and all the mice died within 6 weeks after cell transfer. Five to 6 weeks after the cell transfer, the mice were killed and their colons removed for further analysis. Age- and sex-matched B6 nude mice that had received 5×10^7 lymph node cells from B6 mice and untreated B6 nude mice were used as controls. Four mice were analyzed for each group, and all the experiments were repeated three or four times.

Experimental design

The mice were divided into 4 groups as follows: 1)

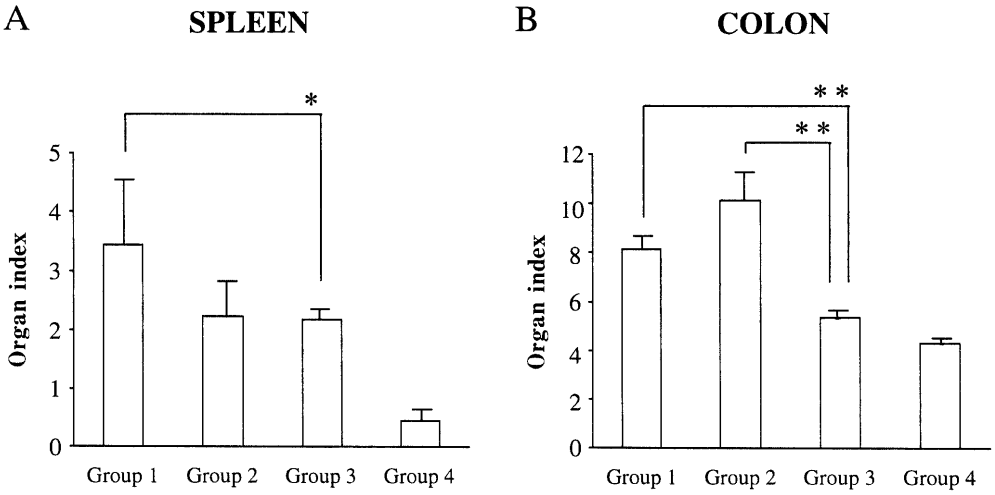

Fig. 1. The effects of a 5-aminosalicyclic acid enema on the systemic and colonic inflammation in MAIDS colitis mice. Spleen index and colon index were calculated as spleen weight (mg)/body weight (g), and colon weight (mg)/body weight (g), respectively. *, $p<0.05$(Student's t test); **, $p<0.01$(Student's t test).

Group 1, MAIDS colitis induced without any enema treatment; 2) Group 2, MAIDS colitis induced and treated with only sodium carboxymethylcellulose (CMC) enema; 3) Group 3, MAIDS colitis induced and treated with 5-ASA granule (0.2 mg/g Body Weight) and CMC enema; and 4) Group 4, B6 nude mice given only CMC enema treatment and used for control. Four mice of each group were killed at 8 weeks after the transfer of lymph node cells of MAIDS mice, and their organs --including the colon-- were taken for analyses.

Evaluation of the effects of 5-ASA enema on the systemic and colonic inflammation in the MAIDS mice

To assess the degree of systemic inflammation and colitis, spleen, liver and colon samples were taken and weighed to calculate each index by the following formula:
Spleen index = spleen weight (mg)/body weight (g)
Liver index = liver weight (mg)/body weight (g)
Colon index = colon weight (mg)/body weight (g)

Monoclonal antibodies

For IF studies, the following monoclonal antibodies were used: anti-CD4 (clone GK1.5, IgG2b), anti-CD8 (clone 53-6.7, IgG2a), anti-B220 (clone RA3-6B2, IgG2a), anti-Mac-1 (clone M-70.15, IgG2b), anti-mouse INF-γ (clone XMG1.2), and anti-mouse IL-10 (clone JES5-16E3).

Histopathological examination

Tissue samples were taken from the colon and rectum, fixed in 10% buffered formalin, and then embedded in paraffin wax blocks. Sections 4 μm thick were made in the usual way and stained with hematoxylin and eosin. The stained sections were then examined by light microscopy.
To assess the degree of colitis, the number of inflammatory cells in a high power field ($\times 400$) were counted under a microscope. Cell numbers at three different points in the lamina propria of the colon of each mouse were counted and the data of three mice from each group were compared statistically.

IF staining procedure

Frozen sections of colon tissue were prepared in a cryostat and stained with several fluorescent dye-conjugated anti-mouse antibodies as described above. The sections were observed by fluorescence microscopy.

Double IF staining procedure

For the simultaneous demonstration of cell surface antigens and cytokines, aceton-fixed frozen sections were incubated sequentially with a biotinylated anti-

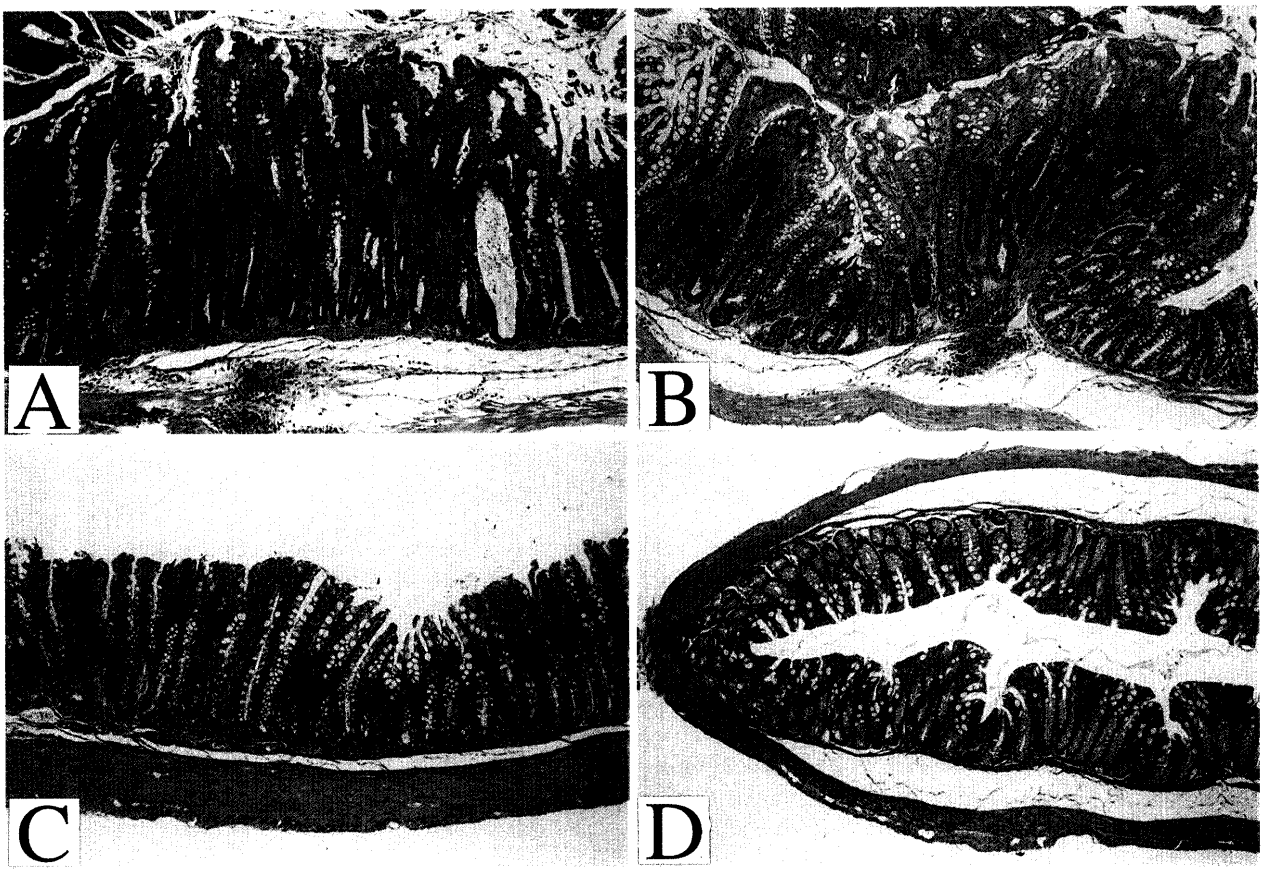

Fig. 2. The effects of a 5-aminosalicyclic acid enema on the ulcerative-colitis lesions of the colon in MAIDS colitis mice. (**A**) B6 nude mice with MAIDS colitis, (**B**) MAIDS mice treated with CMC enema, (**C**) MAIDS colitis mice treated with 5-ASA and CMC enema, (**D**) Control B6 nude mice treated only with CMC enema. (Hematoxyline-eosin; original magnification ×20)

cell surface antigen antibody and then with Alexa-594 (Molecular Probe Inc.)-conjugated avidin as the first step. As the second step, the sections were incubated with fluorescein (FITC)-conjugated anti-cytokine (manuscript describing details of the methods in preparation). The sections were observed by fluorescence microscopy. Controls for the double staining were prepared by omitting the primary antibodies in the first or second step.

Statistical analysis

Data are expressed as means + SE. Statistical analyses were performed using the unpaired Student's *t* test or the nonparametric Mann-Whitney test. Differences were considered significant at $P<0.05$.

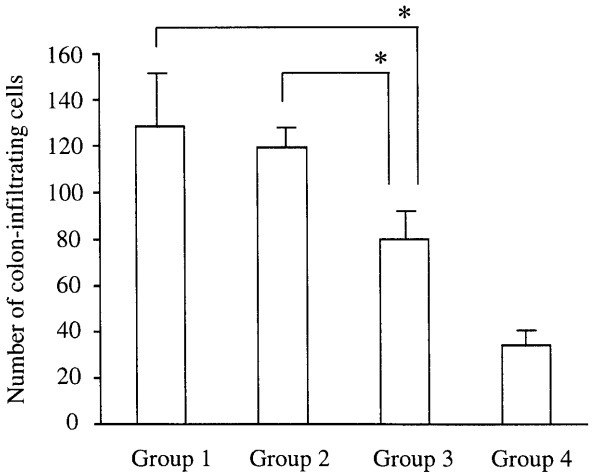

Fig. 3. The effects of a 5-aminosalicyclic acid enema on the number of colon-infiltrating cells in the colon of MAIDS colitis mice. The number of colon-infiltrating cells were counted under high power magnification for three different areas per mouse and the data from the groups were measured with three mice from each and compared each other. *, $p<0.01$ (Student's *t* test).

RESULTS

Clinical course

Nude mice with MAIDS colitis (Group 1) and those treated with CMC enema (Group 2) began to show generalized lymphadenopathy and hepatosplenomegaly at about the 15th day, and runting disease-like symptoms such as body weight loss, lean hunchback, diarrhea, anal prolapse, and rectal bleeding at about the 30th day. MAIDS colitis mice treated with 5-ASA enema (Group 3) showed body weight loss and generalized lymphadenopathy as did the mice of Group 1 and 2, but they showed only mild diarrhea. Irrespective of the presence or absence of treatment, MAIDS colitis mice ultimately died of immunodeficiency at around 50 days after cell transfer. None of these symptoms appeared in the control nude mice treated with a CMC enema (Group 4) throughout the observation period.

Organ weight

As shown in Fig. 1, splenomegaly was observed in nude mice with MAIDS colitis regardless of enema treatment (Group 1, Group 2 and Group 3) compared with the control nude mice (Group 4). In mice with MAIDS colitis, the weight of their colon increased (Group 1, Group 2 and Group 3) compared with the control mice (Group 4); however, this increase was significantly reduced in mice treated with 5-ASA enema (Group 3 vs. Group 1 and 2).

Histopathological analysis

As shown in Fig. 2A, the transfer of MAIDS-cells induced colitis in the recipient nude mice of Group 1. The inflammation termed MAIDS colitis is characterized by mononuclear cellular infiltration into the lamina propria and submucosal layer of the colon, the erosion of colonic epithelial cells, crypt abscess, goblet cell depletion, and hyperplasia of colonic epithelial cells. In the small intestine of mice with MAIDS colitis, cellular infiltration into the lamina propria and submucosal layer were minimal, and neither erosion nor ulceration was observed. These pathological lesions and the dominant involvement of the colon are similar to those of the initial attack of ulcerative colitis. 5-ASA enema treatment effectively ameliorated MAIDS colitis (Group 3, Fig. 2C). In contrast, CMC enema had a marginal therapeutic effect on MAIDS colitis (Group 2, Fig. 2B). No pathological lesions were observed in the colon of CMC enema-treated control mice (Group 4, Fig. 2D). Next, we evaluated statistically the effect of a 5-ASA enema on colitis. The number of cells infiltrating the lamina propria of the colon was also significantly reduced in the mice of Group 3, indicating the efficacy of a 5-ASA enema in treatment for colitis (Fig. 3).

Immunofluorescent analysis

As reported previously, IF studies demonstrated that, as in the colon of mice of Group 1 and Group 2, $CD4^+$ T and Mac-1^+ cells infiltrated diffusely within the lamina propria and that $CD8^+$ T cells were located mainly within the epithelial cell layer of the colon (data not shown). A few $B220^+$ cells were observed in the colon, and in some parts of the mucosa they formed small clusters in the lower part of the lamina propria. These cell surface marker positive cells were rarely observed in mice of Group 3 and 4. IFN-γ-positive cells and IL-10-positive cells were scattered within the lamina propria of the B6 nude mice of Group 1 and 2 (data not shown). On the contrary, these lymphocytes expressing IFN-γ and IL-10 were remarkably reduced in the mice of Group 3 (data not shown). Neither of these cytokines was expressed in the colon of the control mice of Group 4.

To determine the phenotypes of the cells producing these cytokines, we performed double-color IF using both anti-cytokine Abs and anti-cell surface marker Abs. As shown in Fig. 4, $CD4^+$ T cells in the colon of mice of Groups 1 and 2 expressed IFN-γ and IL-10. The number of $CD4^+$ T cells double-positive for IL-10 exceeded that for IFN-γ (Fig. 4A and C). In the 5-ASA enema-treated mice of Group 3, few $CD4^+$ T cells expressed these cytokines (Fig. 4B and D). Mac-1^+ cells in the colon of mice of Groups 1 and 2 expressed IFN-γ and IL-10. The number of Mac-1^+ cells double-positive for IL-10 and that for IFN-γ was almost equal (Fig. 4E and G). There were few Mac-1^+ cells expressing these cytokines in 5-ASA enema-treated mice of Group 3 (Fig. 4F and H). The number of cells double positive for IFN-γ was dominant in Mac-1^+ cells compared with $CD4^+$ T cells. Expression of IL-10 protein was detected on both $CD4^+$ T cells and Mac-1^+ cells. The number of cells double-positive for IL-10 and CD4 exceeded that of cells double-positive for IL-10 and Mac-1. Neither IFN-γ nor IL-10 was detected on $CD8^+$ or $B220^+$ cells (data not shown).

DISCUSSION

5-ASA enemas are well tolerated and are of benefit in the treatment of ulcerative colitis confined to the

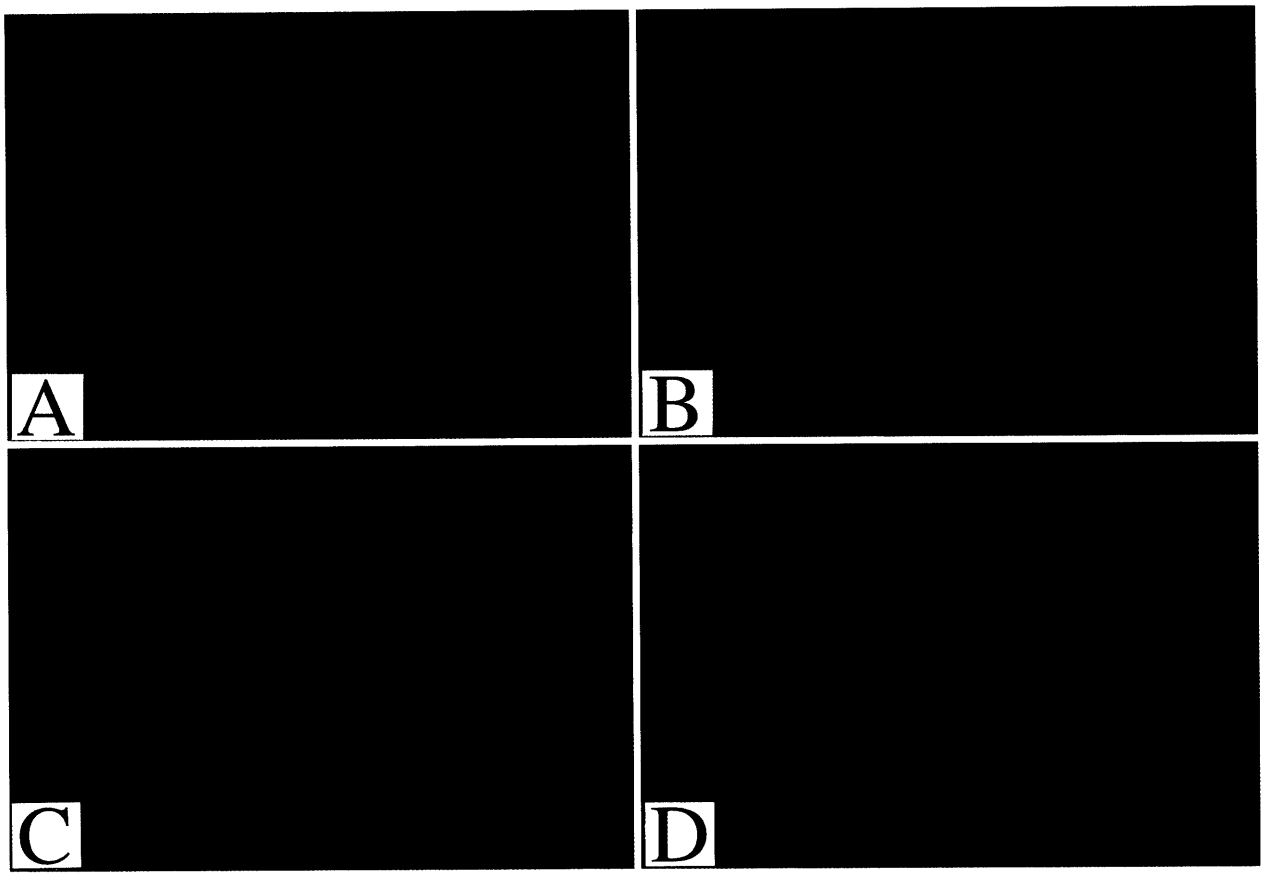

Fig. 4 (A, B, C and D). The effects of a 5-aminosalicyclic acid enema on $CD4^+$ T and $Mac\text{-}1^+$ cells and their expression of IFN-γ or IL-10 in the colon of MAIDS colitis mice. $CD4^+$ T cells were stained with biotinylated anti-CD4 mAb and Alexa 594-conjugated avidin (**A, B, C** and **D**), $Mac\text{-}1^+$ cells were stained with biotinylated anti-Mac-1 mAb and Alexa-594-conjugated avidin (**E, F, G** and **H**). IFN-γ was detected by FITC-conjugated anti-IFN-γ mAb (**A, B, E** and **F**), and IL-10 was detected by FITC-conjugated anti-IL-10 mAb (**C, D, G** and **H**). The colons of mice with MAIDS colitis (Group 1; **A, C, E** and **G**), and those treated with a 5-aminosalicyclic acid enema (Group 3; **B, D, F** and **H**).

distal colon[13,14]. They are reported to be as effective as rectal corticosteroids for curing disease and are better than rectal corticosteroids for inducing remission[15].

Several important actions of 5-ASA have been revealed in neutrophils by inhibiting prostaglandin synthesis, neutrophil migration and degranulation, and the production of reactive oxygen species by neutrophils[12,16]. In addition, 5-ASA inhibits the biosynthesis of LTB_4, one of the arachidonic acid metabolites in the 5-lipoxygenase pathway that are considered an important mediator of inflammation as well as IBD[17]. However, the precise therapeutic mechanism of 5-ASA remains largely unknown.

In this study, we showed that a 5-ASA enema ameliorated the MAIDS colitis. Histological characteristics of MAIDS colitis comprise mononuclear cellular infiltration into the mucosal and submucosal layers of the colon, crypt abscess, and erosion and hyperplasia of the colonic epithelial cells[9,10]. The colon-infiltrating cells are mainly composed of $CD4^+$ T cells and $Mac\text{-}1^+$ macrophages, while neutrophils form a minor population[9,10]. Therefore, the therapeutic effect of a mesalamine enema for MAIDS colitis can not be fully described by its inhibitory effect on neutrophils. It is reasonable to suppose that 5-ASA may inhibit the main effector cells of colitis such as $CD4^+$ T cells and $Mac\text{-}1^+$ cells in MAIDS colitis.

We have shown that the adoptive transfer of $Mac\text{-}1^+$ fraction of MAIDS lymph node cells can induce colitis but not SjS-like exocrinopathy in recipient nude mice[18]. Instead, the transfer of the $CD4^+$ T cell fraction of MAIDS lymph node cells was able to induce SjS-like exocrinopathy but not MAIDS colitis[18].

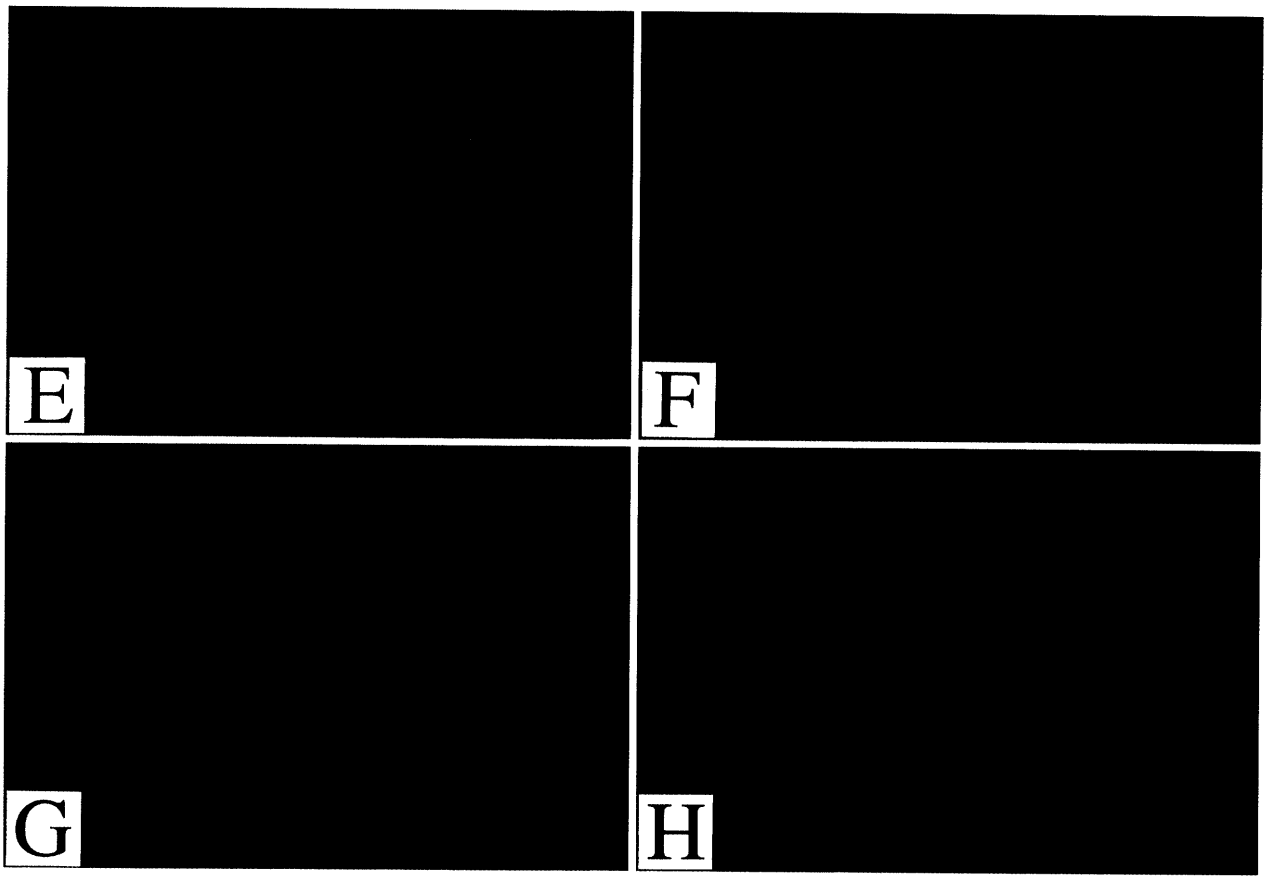

Fig. 4 (E, F, G and H). Legends on the opposite page.

These results suggest that in the development of MAIDS colitis, Mac-1$^+$ cells of MAIDS cells play a principal role. Besides that for neutrophils, 5-ASA might have an inhibitory effect on macrophages. Recently, it has been reported that 5-ASA blocks TNF-α mediated effects on intestinal epithelial cell proliferation and the activation of MAP kinase and NF-$\varkappa$B[20]. In MAIDS colitis, a double IF study revealed that a 5-ASA enema reduced the number of Mac-1 macrophages and CD4 positive T cells that positively stained for IFN-γ or IL-10 in the colon[10]. It is possible that 5-ASA might inhibit the activation of MAP kinase and NF-$\varkappa$B, resulting in the inhibition of the production of these cytokines in macrophages or lymphocytes in MAIDS colitis. We should focus on the pharmacological effects of 5-ASA on these cells as well as neutrophils in a future study.

In our MAIDS colitis, we assumed that immunodeficiency is a systemic symptom induced by CD4$^+$ T cells, and that colitis is a topical symptom which is developed by interaction between Mac-1$^+$ cells, CD4$^+$ cells and gut flora. In this study, a 5-ASA enema exactly showed a beneficial effect on MAIDS colitis; however, the mice eventually died of systemic MAIDS symptoms. It is suggested that 5-ASA is mostly absorbed in the proximal small intestine, and not in the colon, and is predominantly excreted in urine and bile in the acetylated form[20,21]. Therefore, it seems that the 5-ASA enema enabled the effective concentration of 5-ASA in the colon and fully exerted its action topically rather than systemically. In several animal models for IBD, germ-free conditions or the oral administration of antibiotics prevents the development of the colitis. Gut flora is considered the major stimulus to induce or perpetuate chronic intestinal inflammation. It is suggested that 5-ASA may reduce the number of anaerobic bacteria present in the colon. We should further analyze the change in gut flora by a 5-ASA enema to reveal the interaction between the gut flora and induction of MAIDS colitis.

Acknowledgments. We thank Mr. N. Honda for technical assistance. This work was supported by grants from the Ministry of Education and Science and Technology, and

the Ministry of Health, Welfare, and Labor of the Government of Japan.

REFERENCES

1) Sadlack B, Merz H, Schorle H, Schimpl A, Feller AC, Horak I: Ulcerative colitis-like disease in mice with a disrupted interleukin-2 gene. *Cell* **75:** 253-261, 1993.
2) Schull MM, Ormsby I, Kier AB, Pawlowski S, Diebold RJ, Yin M, Allen R, Sidman C, Proetzel G, Calvin D, Annunziata N, Doetschman T: Targeted disruption of the mouse transforming growth factor-beta 1 gene results in multifocal inflammatory disease. *Nature* **359:** 693-699, 1992.
3) Diebold R, Eis MJ, Yin M, Ormsby I, Boivin GP, Darrow BJ, Saffitz JE, Doetschman T: Early-onset multifocal inflammation in the transforming growth factor b1-null mouse is lymphocyte mediated. *Proc Natl Acad Sci USA* **92:** 12215-12219, 1995.
4) Powrie F, Mason D: OX-22high $CD4^+$ T cells induce wasting disease with multiple organ pathology: Prevention by the OX-22 low subset. *J Exp Med* **172:** 1701-1708, 1990.
5) Kuhn R, Lohler J, Rennick D, Rajewsky RN, Muller W: Interleukin-10-deficient mice develop chronic enterocolitis. *Cell* **75:** 263-274, 1993.
6) van Deventer SJ, Elson CO, Fedorak RN: Multiple doses of intravenous interleukin 10 in steroid-refractory Crohn's disease. Crohn's Study Group. *Gastroenterology* **113:** 383-389, 1997.
7) Mosier DE, Yetter RA, Morse HC III: Retroviral induction of acute lymphoproliferative disease and profound immunosuppresion in adult C57BL/6 mice. *J Exp Med* **161:** 766-784, 1985.
8) Suzuki K, Makino M, Okada Y, Kinoshita J, Yui R, Kanazawa H, Asakura H, Fujiwara M, Mizuochi T, Komuro K: Exocrinopathy resembling Sjoegren's syndrome induced by a murine retrovirus. *Lab Invest* **69:** 430-435, 1993.
9) Suzuki K, Narita T, Yui R, Ohtsuka K, Inada S, Kimura T, Okada Y, Makino M, Mizuochi T, Asakura H, Fujiwara M: Induction of intestinal lesions in nu/nu mice induced by transfer of lymphocytes from syngeneic mice infected with murine retrovirus. *Gut* **41:** 221-228, 1997.
10) Suriki H, Suzuki K, Baba Y, Hasegawa K, Narisawa R, Okada Y, Mizuochi T, Kawachi H, Shimizu F, Asakura H: Analysis of cytokine production in the colon of nude mice with experimental colitis induced by adoptive transfer of immunocompetent cells from mice infected with a murine retrovirus. *Clin Immunol* **97:** 33-42, 2000.
11) Khan AK, Piris J, Truelove SC: An experiment to determine the active therapeutic moiety of sulphasalazine. *Lancet* **ii:** 892-895, 1977.
12) Ireland A, Jewell DP: Mechanism of action of 5-aminosalicyclic acid and its derivatives. *Clin Sci* **78:** 119-125, 1990.
13) Sutherland LR, Martin F, Greer S, Robinson M, Greenberger N, Saibil F, Martin T, Sparr J, Prokipchuk E, Borgen L: 5-Amino salicyclic acid enema in the treatment of distal ulcerative colitis, proctosigmoiditis, and proctitis. *Gastroenterology* **92:** 1894-1898, 1987.
14) Safdi M, DeMicco M, Sninsky C, Banks P, Wruble L, Deren J, Koval G, Nichols T, Targan S, Fleishman C, Stat M, Wiita B: A double-blind comparison of oral versus rectal mesalamine versus combination therapy in the treatment of distal ulcerative colitis. *Am J Gastroenterol* **92:** 1867-1871, 1997.
15) Marshall JK, Irvine EJ: Rectal corticosteroids versus alternative treatments in ulcerative colitis: a meta-analysis. *Gut* **40:** 775-781, 1997.
16) Lauristen K, Laursen LS, Bukhave K: Effects of topical 5-aminosalicyclic acid and prednisolone on prostaglandin E2 and leukotriene B4 levels determined by equilibrium in vivo dialysis of rectum in relapsing ulcerative colitis. *Gastroenterology* **91:** 837-844, 1986.
17) Nielsen OH, Bukhave K, Elmgreen J: Inhibition of 5-lipoxygenase pathway of arachidonic acid metabolism in human neutrophils by sulfasalazine and 5-aminosalicyclic acid. *Dig Dis Sci* **32:** 577-582, 1987.
18) Suzuki K, Baba Y, Suriki H, Kawachi H, Shimizu F, Asakura H: Induction of colitis and exocrinopathy in nude mice induced by immunocompetent cell transfer from murine retrovirus-infected mice. In: Asakura H, Aoyagi Y, Nakazawa S (eds) Trends in Gastroenterology and Hepatology. Tokyo: Springer-Verlag Tokyo, 2001, p. 197.
19) Kaiser GC, Yan F, Polk DR: Mesalamine blocks tumor necrosis factor growth inhibition and nuclear factor kB activation in mouse colonocytes. *Gastroenterology* **116:** 602-609, 1999.
20) Paskar BM, Dreyling KW, May B: Possible mode of action of 5-aminosalicyclic acid. *Dig Dis Sci* **32:** S51-56, 1987.
21) Shafil A, Chowdhury JR, Das KM: Absorption, enterohepatic circulation, and excretion of 5-aminosalicyclic acid in rats. *Am J Gastroenterol* **77:** 297-299, 1982.