Cilostazol, a specific PDE-3 inhibitor, ameliorates chronic ileitis via suppression of interaction of platelets with monocytes

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Matsunaga H, Hokari R, Higashiyama M, Kurihara C, Okada Y, Watanabe C, Komoto S, Nakamura M, Kawaguchi A, Nagao S, Miura S. Cilostazol, a specific PDE-3 inhibitor, ameliorates chronic ileitis via suppression of interaction of platelets with monocytes. Am J Physiol Gastrointest Liver Physiol 297: G1077–G1084, 2009. First published October 8, 2009; doi:10.1152/ajpgi.00240.2009—Excessive migration of monocytes to a site of intestinal inflammation contributes to tissue damage in Crohn’s disease. It is known that cilostazol, a specific phosphodiesterase-3 (PDE-3) inhibitor of platelets, decreases monocyte recruitment to intestinal mucosa through suppression of platelet-monocyte interactions. The objective of this study was to clarify whether cilostazol ameliorates murine ileitis by suppression of monocyte migration. Significant inflammation was induced in the ileum of SAMP1/Yit mice at 23 wk of age after piroxicam treatment for 3 wk. Weight of the terminal ileum of mice was significantly greater with inflammatory cell infiltration in SAMP1/Yit mice than in control mice (AKR-J). Treatment of SAMP1/Yit mice with cilostazol-containing food (200 ppm) for 3 wk significantly attenuated the increase in intestinal weight and the histological changes, including invasion of F4/80-positive macrophages. A significant increase in migration of monocytes and platelets to microvessels of the ileal mucosa was observed in SAMP1/Yit mice in vivo by using an intravital fluorescence microscope. Pretreatment with cilostazol significantly attenuated the increased migration of monocytes, possibly through suppression of platelet-monocyte interactions. In conclusion, a PDE-3 inhibitor ameliorates murine ileitis through attenuating migration of monocytes to the intestinal mucosa, suggesting a potential usefulness of antiplatelet drugs for treatment of Crohn’s disease.

The pathogenesis of Crohn’s disease (CD) is not clear, but CD is characterized by an accumulation of inflammatory cells in affected regions of the gastrointestinal tract. Among the various types of inflammatory cells, monocytes and macrophages are thought to play an important role in the development of mucosal inflammation and ulceration (14). Transmural perivascular mononuclear cell infiltrates are a feature of CD, and the vast majority of these cells are CD68+ and CD31+ monocytes/macrophages surrounded by naive and memory T cells (4). It has been suggested that the mechanism of recruitment of monocytes or neutrophils from peripheral blood to tissue involves several steps: rolling, adhesion, and transmigration steps. P-selectin glycoprotein ligand-1 (PSGL-1) plays a significant role in the rolling of monocytes on P-selectin (17, 30).

We previously reported the involvement of PSGL-1 in endotoxin-induced monocyte recruitment to the murine intestinal mucosa (18), and we also demonstrated that blockade of monocyte recruitment to the intestine by using an anti-PSGL-1 antibody successfully ameliorated inflammation in experimental chronic ileitis (17). It has been reported that platelets play a significant role in monocyte recruitment through interaction of PSGL-1 on monocytes with P-selectin on platelets (13). In the rolling step of monocytes, PSGL-1 interacts with P-selectin either on the vascular endothelium directly or on platelets. Since the expression level of P-selectin on activated platelets is 10 times higher than that on the vascular endothelium, monocytes are thought to show efficient rolling in microvessels by interaction with platelets (29). We previously demonstrated that inhibition of platelet activation by using cilostazol, a specific phosphodiesterase-3 (PDE-3) inhibitor that is widely used for an antiplatelet drug, attenuated recruitment of monocytes to inflamed intestinal microvessels after treatment with LPS (15). In intestinal inflammation, contribution of platelets to disease activity has been suggested by studies showing increased platelet activation and aggregation as features of inflammatory bowel disease (IBD) (6) and by the studies showing that numbers of CD40- and CD40L-bearing cells in circulation are increased in IBD patients (7, 8, 9, 24, 40). Thus several attempts have been made to control inflammation by antiplatelet drugs in animal models of gastrointestinal diseases, such as stress-induced gastric injury (33) and ischemia-reperfusion injury of the intestine (16). However, there has been no study to determine whether an antiplatelet drug ameliorates chronic intestinal inflammation, especially focusing on inhibition of monocyte recruitment by blocking platelet-monocyte interaction.

Phosphodiesterases (PDEs) are a diverse family of enzymes that hydrolyze cyclic nucleotides and thus play a key role in the regulation of intracellular levels of the second messengers cAMP and cGMP and hence cell function. Selective PDE inhibitors in a wide range of diseases (sepsis, cardiovascular disease, asthma, schizophrenia, and multiple sclerosis) have been investigated (2, 3, 10, 37). Since PDE-3 is distributed in platelets, a selective PDE-3 inhibitor inhibits activation-dependent α-granule release of P-selectin expressed on the surface of platelets (22) and platelet-leukocyte interaction by reducing CD62P expression on the platelet surface (20). On the other hand, P-selectin expression in the vascular endothelium from Weibel-Palade bodies is regulated by PDE-4. Selective inhibition of PDE-4 decreases P-selectin expression in the vascular endothelium, leading to inhibition of platelet-vascular endothelium interaction (36).
The objectives of this study were 1) to determine whether platelets interact with murine chronically inflamed intestinal microvessels, 2) to determine whether controlling platelet function by PDE-3 inhibitors modulates monocyte interaction with microvessels in the inflamed intestine, and 3) to determine whether PDE-3 inhibitors ameliorate murine ileitis.

METHODS

Animals and induction of ileitis in SAMP/Yit mice. SAMP1/Yit mice were kindly provided by Yakult Central Institute for Microbiological Research, Tokyo, Japan and were maintained in an animal colony at National Defense Medical College, Saitama, Japan. Control AKR/J mice were purchased from Japan Clea, Tokyo, Japan and were maintained in our animal colony. The care and use of laboratory animals were in accordance with the guidelines of the animal facility.

The experimental protocol was approved by the Animal Research Committee of National Defense Medical College. SAMP/Yit mice develop ileitis spontaneously, but the incidence varies among individuals and takes place late in the life. Piroxicam treatment was reported to increase prevalence rate in other spontaneously developed murine models such as IL-10-deficient mice (1) and T cell receptor α chain (TCR-α)-deficient mice (31). To increase prevalence rate and accelerate the development of intestinal disease, mice received piroxicam (200 ppm, Sigma-Aldrich, St. Louis, MO) in standard laboratory chow for 3 wk, from 20 to 23 wk age.

Isolation of monocytes and platelets and labeling with carboxyfluorescein diacetate succinimidyl ester. Monocytes were obtained from bone marrow of thigh bone of AKR/J mice as described previously (6). Mononuclear cell fractions were isolated by centrifugation on a Ficoll-sodium metrizoate density gradient (Ficoll-Paque Plus, GE Healthcare, Chalfont St. Giles, UK) and suspended in medium. Monocytes were isolated by magnetic cell sorting system (MACS, Miltenyi Biotec, Auburn, CA) with bead-conjugated rabbit anti-mouse CD11b polyclonal antibody (Miltenyi Biotec). The purity of monocytes was evaluated by a fluorescence-activated cell sorter.

Fig. 1. Ratio of rolling platelets in intestinal microvessels 20 min after infusion of labeled platelets. Platelets were stained with carboxyfluorescein diacetate succinimidyl ester CFDSE ex vivo and injected via cervical vein. The rolling ratio is expressed as percentage of rolling cells among the total cell influx. AKR/J, control AKR/J mice; SAMP/Yit, piroxicam-treated SAMP/Yit mice (disease control); anti-P-selectin, treatment with anti-P-selectin antibody to SAMP/Yit mice; cilostazol, pretreatment with cilostazol-containing diet to SAMP/Yit mice. †P < 0.05 vs. control AKR/J mice. ‡P < 0.05 vs. piroxicam-treated SAMP/Yit mice.

Fig. 2. Representative photos of in vivo observation of platelet adhesion in intestinal microvessels. In control AKR/J mouse, a small number of CFDSE-labeled platelets adhered to submucosal venules (A). In piroxicam-treated SAMP/Yit mouse, the number of adherent platelets significantly increased (B). In piroxicam-treated SAMP/Yit mouse, antibody against P-selectin treatment significantly attenuated the increased number of adherent platelets (C). Bars indicate 100 μm.

Fig. 3. Ratio of rolling monocytes in intestinal microvessels 20 min after infusion of labeled monocytes. Monocytes were stained with CFDSE ex vivo and injected via cervical vein. †P < 0.05 vs. control AKR/J mice. ‡P < 0.05 vs. piroxicam-treated SAMP/Yit mice (disease control).

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each group. P‡ cosal venules significantly increased. The number of adhered monocytes to inflamed submucosal venules was observed from the serosal side by use of an intravital microscope. The behavior of CFDSE-labeled monocytes and platelets was visualized on a monitor through a silicon-intensified target image tube system, by a previously described method, and recorded on digital hard disk recorder (18). Microcirculation was observed by a fluorescence microscope (BX51WI, Olympus, Tokyo, Japan) equipped with a contrast-enhancing unit (C-2400-08, Hamamatsu Photonics, Shizuoka, Japan) and ×10 ultraviolet-fluorite objective lens (UPlan Fl, Olympus). Migration behaviors between AKR/J mice and SAMP/Yit mice were compared. In some experiments, 2 mg/kg of rat anti-mouse P-selectin (RB40. 34; BD PharMingen, San Diego, CA).

Platelets were isolated from blood of donor mice as described previously (19, 41). Blood of mice was collected from the heart and platelets were isolated by centrifugation method at 600 g with 0.1 ml acid citrate dextrose buffer. The expression of P-selectin on platelets was compared between platelets in whole blood and isolated platelets by the centrifugation method with a fluorescence-activated cell sorter using rat anti-mouse P-selectin (RB40. 34; BD PharMingen, San Diego, CA).

Carboxyfluorescein diacetate succinimidyl ester (CFDSE; Molecular Probes, Eugene, OR) was dissolved in dimethylsulfoxide at 15.6 mM, divided into small aliquots (each 300 μl), and stored in a cuvette sealed with argon gas at −20°C until use in the experiments. Monocytes (≈2×10⁷) in 1.5 ml of PBS were incubated with CFDSE solution for 10 min at 4°C and washed by PBS. Platelets (≈1×10⁸) were incubated with CFDSE solution for 10 min at 4°C and washed by PBS.

Animal preparation for intravital observation. For cell recruitment studies, recipient mice of 23 wk of age were anesthetized with 50 mg/kg pentobarbital sodium, and the abdomen of each mouse was opened with a midline incision. An ileal segment of 1–3 cm in length was selected for observation. The intestine was kept warm and moist by continuous superfusion with PBS warmed to 37°C. PBS was injected into the selected segment via a 30-gauge needle. The behavior of monocytes and platelets in submucosal venules was observed from the serosal side by use of an intravital microscope. The behavior of CFDSE-labeled monocytes and platelets was visualized on a monitor through a silicon-intensified target image tube system, by a previously described method, and recorded on digital hard disk recorder (18). Microcirculation was observed by a fluorescence microscope (BX51WI, Olympus, Tokyo, Japan) equipped with a contrast-enhancing unit (C-2400-08, Hamamatsu Photonics, Shizuoka, Japan) and ×10 ultraviolet-fluorite objective lens (UPlan Fl, Olympus), and fluorescence mirror unit (UIS2, Olympus).

Analysis of monocyte and platelet dynamics. The number of rolling and adherent cells was counted offline by digital video disk images over a period of 40 min after the injection. Monocytes and platelets adhering to vascular walls with or without occasional movements were defined as rolling cells. The rolling percentage was expressed as the ratio of the number of rolling cells divided by the number of total cell influx at the 20 min after infusion. The monocytes or platelets adhering to vascular walls without movement and remaining stationary for a period of more than 30 s were defined as adherent cells. We counted the number of adherent cells in the field of 1 mm².

Effect of cilostazol treatment on ileitis of SAMP1/Yit mice (treatment study). We next examined whether administration of cilostazol ameliorates ileitis of SAMP1/Yit mice. Various concentrations of cilostazol ranging from 0.02% (200 ppm) to 0.2% in diet was used for animal study (23, 43). Because we chronically treated mice with cilostazol, we chose the lowest concentration. From 20 to 23 wk of age for 3 wk, mice received food containing 200 ppm of cilostazol. After 3 wk of treatment, mice were euthanized and terminal ileum was
removed at 23 wk of age, and weight of the last 7-cm segment of terminal ileum was measured. Histological changes were evaluated in hematoxylin and eosin-stained sections in a blinded fashion. Total thickness and thickness of proper muscularis of the terminal ileum were measured and averaged in proportion with the length of intestine.

**Immunohistochemistry.** Localization and expression of CD3 and F4/80 in the intestinal mucosa were assessed by immunohistochemistry by the labeled streptavidin-biotin method. The terminal ileum of SAMP1/Yit mice and age-matched AKR/J mice were removed and fixed in periodate-lysine-paraformaldehyde solution and were vertically embedded in optimum cutting temperature compound (Miles, Elkhart, IN) before being frozen in dry ice and acetone. Well-orientated cryostat sections of 6 μm in thickness were transferred to poly-l-lysine-coated slides and air dried for 1 h at room temperature. After they were washed in PBS containing 1% Triton X (LKB-Produkter, Bromma, Sweden) for 5 min, sections were incubated in 10% normal goat serum in PBS. Monoclonal antibodies used in this study were as follows: anti-mouse CD3 antibody (17A2; BD PharMingen), anti-mouse F4/80 antibody (53/6; BD PharMingen). Isotype matched IgG was used as controls. They were diluted 50–100 times with PBS and layered on the section overnight at 4°C. Sections were incubated with second antibody, biotinylated anti-rat IgG class antibody (BD PharMingen) for 1 h at room temperature. Then sections were incubated with FITC-conjugated streptavidin (streptavidin-fluorescein) (Amersham Biosciences, Buckinghamshire, UK) for 30 min at room temperature. Rinsing with PBS was performed between each step. A coverslip was applied by using glycerol jelly. These sections were observed under a confocal microscope (Carl Zeiss, Oberkochen, Germany). The infiltrating cells were expressed as the numbers of CD3 and F4/80-positive cells per millimeter of muscularis mucosa.

Fig. 6. Histological changes of the ileum as determined in hematoxylin and eosin-stained sections. Control AKR/J (×20 objective; A) and SAMP1/Yit mice (B) at 23 wk show inflammation, characterized by distorted villous architecture, expansion of lamina propria, and thickening of muscularis propria (×20). C: 3-wk treatment with cilostazol to SAMP1/Yit mice with piroxicam treatment. Histological determination of submucosal thickness (D) and thickness of muscular layer (E) in control AKR/J mice (open bars), SAMP1/Yit mice with piroxicam (diseased control) (solid bars), and cilostazol-treated SAMP1/Yit mice with piroxicam (shaded bars). F: weight of terminal ileum per 7-cm length. Values are expressed as means ± SD. ††P < 0.01 vs. control AKR/J mice, ‡P < 0.05 vs. piroxicam-treated SAMP/Yit mice (disease control), ‡‡P < 0.01 vs. piroxicam-treated SAMP/Yit mice (disease control).
Statistics. All results were expressed as means ± SD. The data were analyzed by one-way ANOVA with Fisher’s post hoc test. Statistical significance was set at \( P < 0.05 \). Student’s \( t \)-test was used when only two groups were compared. Statistical significance was set at \( P < 0.05 \).

RESULTS

Enhanced platelet interaction with microvessels of SAMP/Yit mice and the effect of cilostazol. We first investigated whether rolling of platelets increases chronically inflamed intestinal microvessels in SAMP/Yit mice after 3 wk of piroxicam treatment. Figure 1 shows the percentage of rolling platelets at 20 min after infusion. The total number of platelets that had entered small intestinal microvessels at 20 min did not differ among the three groups (control AKR: 49 ± 8.1/min, SAMP/Yit small intestine: 51.5 ± 7.2/min, SAMP/Yit with cilostazol treatment: 47.3 ± 7.2/min). About 20% of platelets showed rolling behavior in control AKR/J mice. The rolling ratio was ~40% in inflamed intestines of piroxicam-treated SAMP/Yit mice, and it was significantly higher than that in AKR/J mice, suggesting the involvement of platelets in the pathophysiology of ileitis. Pretreatment with anti-P-selectin antibody significantly attenuated the increased ratio of rolling in piroxicam-treated SAMP/Yit mice. On the other hand, pretreatment with cilostazol did not attenuate the increased ratio of rolling in SAMP/Yit mice.

Only a few platelets adhered to the vascular endothelium of intestinal microvessels in control AKR/J mice (Fig. 2A). Adhesion of platelets was significantly enhanced in SAMP/Yit mice (Fig. 2B). The enhanced platelet adhesion in SAMP/Yit mice was remarkably attenuated by anti-P-selectin antibody (Fig. 2C) but was not significantly reduced by cilostazol (data not shown). These findings indicate that P-selectin is involved in platelet adhesion to chronically inflamed intestinal microvessels.

Effects of cilostazol on monocyte recruitment to chronically inflamed intestinal microvessels in SAMP/Yit mice. Next we investigated whether interaction of monocytes with chronically inflamed intestinal microvessels is enhanced in piroxicam-treated SAMP/Yit mice and how cilostazol affects monocyte interactions (Figs. 3 and 4). Recipient mice were injected with CFDSE-labeled monocytes and migration behavior in the small intestine was observed via a fluorescence microscope. The total number of monocytes that had entered small intestinal microvessels at 20 min did not differ among the three groups (control AKR 86 ± 7.7/min, SAMP/Yit 97 ± 11/min, SAMP/Yit with cilostazol 82 ± 9.7/min). Rolling rates of monocytes was determined at 20 min after monocyte infusion (Fig. 3). In AKR/J mice, ~35% of monocytes interacted with microvessels. A larger number of monocytes showed interaction with intestinal microvessels in piroxicam-treated SAMP/Yit mice than in AKR/J mice, and the rolling ratio of monocytes in-
creased significantly. Pretreatment with anti-P-selectin antibody drastically attenuated the monocyte rolling with intestinal microvessels of SAMP/Yit mice. Pretreatment with cilostazol also significantly attenuated the rolling of monocytes to levels comparable to those for control AKR mice.

Figure 4 shows the time course of changes in monocyte adhesion to intestinal microvessels. The number of monocytes that adhered to intestinal microvessels of piroxicam-treated SAMP/Yit mice time dependently increased after cell infusion and the number was significantly greater than that in AKR mice after 20 min. Cilostazol treatment almost completely inhibited the increase in number of monocytes adhering to intestinal microvessels in SAMP/Yit mice.

Interaction of platelets and monocytes with intestinal microvessels. To evaluate interaction between platelets and monocytes in the rolling and adhesion process, simultaneous observation of them by individual staining was performed. Figure 5 shows the interaction between platelets and monocytes observed by a 3CCD camera. In control AKR mice, few monocytes and few platelets showed rolling behavior (Fig. 5A). In SAMP/Yit mice, both platelets and monocytes showed enhanced migration behavior. Some platelets directly interacted with the vascular endothelium (platelet-endothelial interactions), whereas other platelets adhered to monocytes (platelet-monocyte interactions), and the platelets bearing monocytes rolled and adhered to the endothelium of microvessels (Fig. 5B). In cilostazol-treated SAMP/Yit mice, platelet-endothelial interactions were not significantly changed; however, the number of rolling monocytes significantly decreased and platelet-monocyte interactions significantly decreased by this treatment (Fig. 5C).

Effect of cilostazol treatment on development of ileitis in SAMP1/Yit mice. Next we investigated the effect of cilostazol treatment on the development of ileitis. After 3 wk of piroxicam treatment, 100% of SAMP/Yit mice developed moderate to severe ileitis. We therefore used this model as a disease control to evaluate the effect of cilostazol treatment. Histological changes and intestinal weight in the ileum are shown in Fig. 6. Characteristic features of ileitis, including decreased villus height, submucosal thickening, and increased size of the muscular layer, were observed in the disease control SAMP/Yit mice but not in the control AKR/J mice (Fig. 6, A and B). Cilostazol treatment significantly ameliorated ileitis as shown in Fig. 6C. Figure 6, D and E, shows total thickness and thickness of the proper muscularis of the ileum. SAMP/Yit mice showed significantly greater thicknesses than those in AKR mice. Cilostazol also significantly attenuated these parameters in SAMP/Yit mice. A comparison of the weights of terminal ileum showed that the weight was significantly heavier in SAMP1/Yit mice than in AKR/J mice, and cilostazol also effectively attenuated the increase in intestinal weight (Fig. 6F).

Effect of cilostazol treatment on leukocyte infiltration in the intestinal mucosa of SAMP/Yit mice. The numbers of F4/80-positive cells (Fig. 7, A–C) and CD3-positive cells (Fig. 7, D–F) infiltrating the intestinal mucosa was investigated by immunohistochemical study. Only a small number of leukocytes infiltrated the intestine in AKR/J mice (Fig. 7, A and D). A marked increase in both the number of F4/80-positive cells (Fig. 7B) and number of CD3-positive cells (Fig. 7E) infiltrating the intestine was observed in piroxicam-treated SAMP/Yit mice. Treatment with cilostazol for 3 wk significantly attenuated the increase in infiltration of both F4/80-positive cells (Fig. 7C) and CD3-positive cells (Fig. 7F). The number of infiltrating leukocytes per intestinal length is shown in Fig. 8. Cilostazol treatment significantly attenuated the increase in both CD3-positive cells (Fig. 8A) and F4/80-positive cells (Fig. 8B).

DISCUSSION

In this study, we showed that 1) recruitment of monocytes to inflamed ileal microvessels of SAMP/Yit mice was enhanced in a P-selectin-dependent fashion; 2) cilostazol attenuated the increase in recruitment of monocytes to the ileal microvessels of SAMP/Yit mice, possibly by suppression of platelet-monocyte interaction; and 3) cilostazol ameliorated ileitis in SAMP/Yit mice with decrease in monocyte infiltration. Taken to-
gether, the results suggest that cilostazol is a promising drug for treatment of IBD.

Blocking of leukocyte recruitment to the intestinal mucosa is one of the major strategies for IBD treatment. We previously reported that control of T cell recruitment by using an antibody against MAAdCAM-1 ameliorated ileitis in the same SAMP/Yit mice (28). In clinical studies, therapeutic compounds directed against trafficking of leukocytes have been designed and are being developed as a novel class of drugs for treatment of inflammatory diseases, especially CD (35, 42). Although these biological modifiers have promising effects, there are relatively high risks of side effects such as anaphylaxis known as infusion reaction, and formation of a human antichimeric antibody is responsible for decrease in long-term efficacy. In addition, possible associations between biological modifiers and inurable diseases, such as hepatosplenic T cell lymphoma and progressive multifocal leukoencephalopathy, have been reported (5, 34). Thus drugs to control leukocyte trafficking not by biological modification may have some beneficial roles for controlling IBD especially in terms of safety.

Anti-inflammatory effects of cilostazol, a specific PDE-3 inhibitor widely used as an antiplatelet drug, have been reported in several animal models of inflammation, such as gastritis (32), diabetic renal injury (44), and nonalcoholic fatty liver diseases (11), although its exact mechanism of action has not been elucidated. In this study, we showed that the beneficial effect of cilostazol on ileitis may be largely due to inhibition of monocyte/macrophage infiltration via monocyte-platelet interaction. This blocking of monocyte-platelet interaction by cilostazol is thought to be due to its effect on platelets but not on monocytes, because monocytes do not have PDE-3 but rather PDE-2. IC50 of cilostazol are 0.20 µM for PDE-3 and 45.2 µM for PDE-2 (39). PDE-3 is distributed to platelet, heart, vascular smooth muscle, oocytes, and hepatocytes but not to monocyte/macrophages (25). Thus effect of cilostazol on monocyte function is thought to be small in this study. In addition to lymphocytes, monocytes/macrophages are also involved in inflammation of IBD. In a physiological state, a certain number of macrophages reside in the intestinal mucosa and play an anti-inflammatory role in keeping the intestinal mucosa uninflamed. However, in an inflamed condition, it is thought that proinflammatory macrophages/macrophages are recruited from the bloodstream. These recruited macrophages are phenotypically different from the resident population of cells and play a major role in mediating the chronic mucosal inflammation by producing a larger amount of IL-23 or TNF-α (21, 26).

Previously, we studied the effect of antiadhesion molecule antibody treatment for monocyte recruitment on ileitis of SAMP/Yit mice, which are the same mice as used in this study (17). In vivo intravital microscopy observation showed that anti-PSGL-1 antibody treatment blocked monocyte migration to small vessels of inflamed intestinal mucosa in SAMP/Yit mice. Long-term anti-PSGL-1 antibody treatment significantly attenuated severity of ileitis with decrease in number of monocyte infiltration. These results suggest that SAMP/Yit mice are a good model for studying the effect of controlling monocyte recruitment on development of ileitis and that control of monocyte recruitment would be an efficient strategy for IBD treatment (17).

Recently, it has been reported that platelets are involved in adhesion of monocytes to the atherosclerotic endothelium through platelet-monocyte interaction (38). Platelet adhesion to the endothelium preceded atherosclerotic plaque invasion by leukocytes, and blockade of platelet adhesion greatly reduced atherosclerotic lesion formation (27). In gastrointestinal disease, it has also been proposed that platelets are involved in activity of IBD and has been suggested that the effect of leukocytapheresis is in part due to removal of platelets in addition to leukocytes (12).

In this study, an antibody against P-selectin strongly inhibited the migration of not only monocytes but also platelets by blocking P-selectin expression on the endothelium as well as on platelets. On the contrary, because cilostazol selectively attenuates P-selectin expression on platelets but not on the endothelium, we confirmed that cilostazol only inhibited the adherence of monocytes to intestinal microvessels without affecting the direct interaction of platelets with intestinal microvessels. Although the blocking effect of cilostazol on monocyte rolling was weaker than that by the antibody against P-selectin, the increase in monocyte adhesion was reduced to almost the control AKR levels. Collectively, we can suggest that the antiplatelet action of cilostazol is sufficient to prevent the development of ileitis and that cilostazol is safer in terms of risk of gastrointestinal bleeding than is anti-P-selectin antibody, because it does not directly block platelet-endothelial interaction.

In this study, long-term cilostazol treatment decreased not only infiltration of monocytes but also that of lymphocytes. We previously studied the effect of an antibody to PSGL-1, which is exclusively expressed on monocytes, on SAMP/Yit mice. PSGL-1 antibody treatment inhibited lymphocyte infiltration as well as monocyte infiltration to the inflamed mucosa of SAMP/Yit mice, suggesting that the role of monocytes in SAMP/Yit mouse ileitis is more critical than that of lymphocytes (17). Thus we consider that the inhibitory effect of cilostazol on lymphocyte migration was not direct but secondary to monocyte recruitment inhibition.

Finally, we showed the possible involvement of monocytes/macrophages and platelets in ileitis of SAMP/Yit mice and that an antiplatelet drug, cilostazol, has the capacity to ameliorate ileitis. Ileitis in CD is still difficult to cure; however, the widely used drug cilostazol might become an effective choice of treatment for small intestinal CD.

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DISCLOSURES

There is no personal or financial conflict of interest to disclose for any of the author listed.

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