Proteinase-activated Receptor-1 is an Anti-inflammatory Signal for Colitis Mediated by a Type 2 Immune Response

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Background: Activation of colonic proteinase activated receptor-1 (PAR_1) provokes colonic inflammation and increases mucosal permeability in mice. The mechanism of inflammation is not neurogenic like in the paw of rats but depends on PAR_1-mediated activation monocytic cells. PAR_1 activation in the colon increases the release of lymphocyte T helper-1 (T_H1) cytokines. Moreover, PAR_1 expression is increased in biopsies from patients with inflammatory bowel disease, and its activation during T_H1-mediated colitis in mice increases all of the hallmarks of inflammation.

Methods: This study aimed to characterize the effects of PAR_1 activation in oxazolone-mediated colitis, involving a T_H2 cytokine profile.

Results: Intracolonic administration of oxazolone increased myeloperoxidase activity, damage score, and interleukin (IL)-4, IL-10, tumor necrosis factor α, and IL-1β mRNA expression but lowered interferon-γ mRNA expression, indicating colonic inflammation of a T_H2 profile. The concurrent intracolonic administration of a PAR_1 agonist in oxazolone-treated mice inhibited colitis, resulting in a reduction of myeloperoxidase activity, damage score, and inflammatory cytokine mRNA expression. Using PAR_1-deficient mice, we confirmed that the anti-inflammatory effects of PAR_1 agonists were mediated by PAR_1. Moreover, in PAR_1-deficient mice or in mice treated with a PAR_1 antagonist, oxazolone-induced colitis was exacerbated, showing an endogenous modulatory role for PAR_1 in this T_H2 cytokine profile of colitis.

Conclusions: Thus, as opposed to a previously shown proinflammatory role for PAR_1 in a T_H1 cytokine-mediated colitis, our new data show anti-inflammatory role for PAR_1 activation in the setting of T_H2 cytokine colitis model.

Key Words: colon, inflammation, mucosa, proteinase-activated receptor-1, rodent, T helper-1/T helper-1 cells

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Proteinase activated receptor-1 (PAR_1) belongs to a novel family of 7 transmembrane domain G-protein–coupled receptors that are activated by cleavage of their N-terminal domain by a proteolytic enzyme.[1] The unmasked new N-terminal sequence acts as a tethered ligand that binds and activates the receptor itself. PAR_1 is activated by several endogenous proteases, such as thrombin, the coagulation factor Xa, and granzyme A[2], and by exogenous proteases, such as gingipain-R, an arginine-specific proteinase released by the mouth pathogen *Porphyromonas gingivalis*.[3] Others proteases such as plasmin, elastase, cathepsin G, and proteinase 3 can "disarm" PAR_1 by removing its N-terminal activation site.[2] Synthetic peptides, so-called PAR-activating peptides (PAR-APs; TFLLR-NH_2 for PAR_1 [TFLLR]), based on the tethered ligand sequence, are also able to selectively activate PARs, providing useful pharmacological tools to understand the physiology of these receptors.[4] PAR_1 was the first member of this family to be cloned and was the first receptor for which this unique mechanism of activation was described.[5] The interest in PAR_1 was initially driven by research on the receptor responsible for thrombin-induced platelet activation. However, PAR_1 activation also induces changes in vascular tone, increased vascular permeability, and granulocyte chemotaxis.[6,7] In vitro, PAR_1 activation directed migration of human eosinophils and thereby may affect eosinophils in tissue and allergic inflammation.[8] In lung epithelial cells, PAR_1 agonists stimulated the release of proinflammatory cytokines (interleukin [IL]-6 and IL-8).[9] In vivo, a recent study has shown that activation and up-regulation of PAR_1 could contribute to brain inflammation and neuronal damage during HIV-1 infection.[10] Moreover, in rat paws, thrombin was implicated in both phases of carrageenan-induced edema.[11] In that model, thrombin acts both by stimulating the release of

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bioactive amines from mast cells and by triggering polymorphonuclear cell chemotaxis.[11] However, although PAR_1 agonists at different concentrations reproduced the effect of thrombin in carrageenan-induced edema,[11,12] PAR_1 selective agonists failed to reproduce the effects of thrombin on leukocyte recruitment.[13] In the gut, PAR_1 is expressed by a variety of cell types, including enterocytes; endothelial cells; enteric neurons, where it coexpresses with excitatory and inhibitory neurotransmitters;[14] mesenteric afferent nerves terminals; myocytes; and immune cells such as monocytes/macrophages, neutrophils, and lymphocytes.[15] Both in vivo and in vitro PAR_1 agonists induce apoptosis of intestinal epithelial cells in a caspase-3–dependent manner, with a concomitant loss of the epithelial barrier function and a consequent increase of permeability to macromolecules and bacteria.[16] In a previous study, we have shown (1) that PAR_1 expression was upregulated in biopsies from patients with inflammatory bowel disease (IBD), (2) that PAR_1 activation in the colon of mice led to an inflammatory reaction involving lymphocytes, and (3) that PAR_1 activation played a role in the pathogenesis of 2 experimental models of IBD.[17] The inflammatory role of PAR_1 in these IBD models involved, secondarily, an increase in intestinal permeability, and, primarily, the activation of monocytic cells from the lamina propria, leading to cytokine release. PAR_1 activation per se was observed to trigger a T helper-1 (T_H1) cytokine profile. Furthermore, in mice, the increase in interferon (IFN)γ and IL-2 (2 T_H1 profile cytokines) observed in response to trinitrobenzene sulfonic acid (TNBS)-induced colitis was inhibited by pretreatment with a PAR_1 antagonist.[17]

Considering the induction of T_H1 cytokines observed on PAR_1 activation in the gastrointestinal tract, we hypothesized that PAR_1 activation in the intestine may counteract the release of T_H2 cytokines, thereby inhibiting inflammation with a T_H2 profile. We therefore evaluated the impact of PAR_1 activation on the development of T_H2 colitis (oxazolone-induced colitis). In a second set of experiments, we compared the colonic inflammatory response induced by oxazolone administration in wildtype and $PAR_1^{-/-}$ mice and in mice treated with a PAR_1 antagonist. Finally, we evaluated the cytokine profile triggered by PAR_1 activation during the course of colitis induced by oxazolone. Our results show that PAR_1 exerts anti-inflammatory properties in oxazolone-induced colitis by switching the cytokine balance toward a T_H1 cytokine profile.

MATERIALS AND METHODS

Animals

C57BL/6 mice were obtained from Charles River Laboratories (Quebec, Canada). $PAR_1^{-/-}$ mice and littermates (C57BL/6 background) were originally provided by Johnson & Johnson Pharmaceutical Research and Development and bred at the University of Calgary. All mice were housed in a temperature-controlled room and had free access to food and water. The Animal Care Committee of the University of Calgary approved all experimental protocols.

Chemicals

Peptides (the selective PAR_1-activating peptide TFLLR-NH_2 and the control peptide RLLFT-NH_2, which is inactive on PAR_1) were obtained from the Peptide Synthesis Facility of the Faculty of Medicine of the University of Calgary (Peplab@ucalgary.ca). The PAR_1 antagonist used in this study (SCH 79797) was purchased from Tocris (Ellisville, Mich.).[18,19] Oxazolone was purchased from Sigma-Aldrich (Ontario, Canada).

Reverse Transcriptase-Polymerase Chain Reaction

For reverse transcriptase-polymerase chain reaction (RT-PCR) performed in mouse tissues, total RNA was isolated using TRIzol reagent (Invitrogen Corp., Ontario, Canada) according to the instructions of the manufacturer. The sense and antisense primers, respectively, were as follows: β-actin: 5′-GGGTCAGAAGGATTCCTATG-3′ and 5′-GGTCTCAAACATGATCTGGG-3′; IL-10: 5′-ATGCAGGACTTTAAGGGTTACTTG-3′ and 5′-AGACACCTTGGTCTTGGAGCTTA-3′; IL-4: 5′-TCGGCATTTTGAACGAGGTC-3′ and 5′-GAAAAGCCCGAAAGAGTCTC-3′; IFN-γ: 5′-GCTCTGAGACAATGAACGCT-3′ and 5′-AAAGAGATAATCTGGCTCTGC-3′; tumor necrosis factor-α (TNFα): 5′-TCTCATCAGTTCTATGGCCC-3′ and 5′-GGGAGTAGACAAGGTACAAC-3′; IL-1β: 5′-AGAAGGTGCTCATGTCCTCAT-3′ and 5′-TTGACGGACCCCAAAAGATG-3′.

The PCR reaction was performed using 2 μL of cDNA. This product was amplified in a final concentration of 1 PCR buffer (Perkin Elmer/Cetus, Quebec, Canada), 0.8 μmol/L of each primer, 0.2 mmol/L of deoxy-nucleotides tri-phosphate (dNTPs), and 1 U of Taq polymerase (Perkin Elmer/Cetus) in a total volume of 50 μL. Semiquantitative PCR used the "primer-dropping" method, in which β-actin was coamplified as an internal control in all reactions as described. Aliquots of PCR reactions (~20 μL) were equalized to the equivalent signals from the β-actin mRNA and separated by electrophoresis through 1% agarose gels containing 0.2 μg/mL ethidium bromide. Gels were photographed and analyzed by densitometry using NIH imaging software. PAR_1 mRNA expression is presented as a ratio to β-actin.

Colitis Induction and Study Design

Mice who fasted for 12 hours were lightly anesthetized with halothane, and a polyethylene catheter was inserted intrarectally 4 cm from the anus. All compounds given intracolonically were administered through the catheter at a maximum volume of 100 μL, as previously described. Peptides (TFLLR-NH_2 and RLLFT-NH_2) were dissolved in 10% ethanol, 10% Tween-80, and 80% saline (0.9% NaCl). After the intracolonic administration of the peptides or their vehicle, mice (8 in each

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group) were killed, and distal colonic tissues were harvested. For all studies macroscopic-damage score was assessed as previously described, scoring inflammatory parameters such as erythema, hemorrhage, edema, stricture formation, ulceration, presence of blood in the feces, mucus presence, diarrhea, and adhesions.[17] Myeloperoxidase (MPO) activity was assessed as an index of granulocyte infiltration by an observer unaware of the treatments,[20] and the weight and the survival rate of mice were recorded.

On day 0, mice (wildtype and $PAR_1^{-/-}$) were swabbed on the abdomen with a mixture of 3% oxazolone in olive oil (oxazolone is 4-ethoxymemethylene-2-phenyl-2-oxazolin-5-one; Sigma) to sensitize them. At day 7, mice were given an intracolonic injection of 1% oxazolone in a solution of 50% ethanol and 50% saline or only vehicle. At day 11, mice were killed, and pieces of the colon approximately 2 cm from the anus were taken for MPO activity, RT-PCR, and histology. For the experiments aimed at determining whether or not PAR_1 activation reduced colitis, the PAR_1-activating peptide TFLLR-NH_2 (TFLLR) or the control peptide RLLFT-NH_2 (each at the dose of 50 μg/mouse) was administrated intracolonically or intraperitoneally 1 hour before and daily after intracolonic administration of oxazolone or its vehicle. Body weight was recorded daily as an index of disease progression, and the same inflammatory parameters (macroscopic-damage score, MPO) were assessed 4 days after intracolonic oxazolone administration, at the time of death. For the studies performed with the PAR_1 antagonist, mice underwent the same oxazolone treatment, and the PAR_1 antagonist (5 mg/kg) or its vehicle (carboxymethyl cellulose) were administered intraperitoneally daily from day 7 (1 h before oxazolone administration) until day 11. As previously described, on day 11, the mice were killed, distal colonic tissues were harvested, and the same inflammatory parameters were measured.

RESULTS

Effects of PAR_1-Activating Peptide (PAR_1-AP) Administration on Oxazolone-Mediated Colonic Inflammation

Sensitization and subsequent challenge by intracolonic administration of oxazolone induced colitis as measured by an increase in MPO activity, damage score, and colonic wall thickness and induced a severe wasting syndrome (Fig. 1, A–D). Activation of PAR_1 by the agonist peptide, TFLLR, administrated intracolonically 1 hour before and daily after intracolonic oxazolone administration significantly reduced oxazolone-induced increase in MPO activity (4.50 ± 1.60 versus 24.80 ± 10.40 U/mg of tissues; $P < 0.05$), damage score (0.83 ± 0.50 versus 2.90 ± 0.60; $P < 0.05$), and wall thickness (0.44 ± 0.05 versus 0.61 ± 0.08 mm; $P < 0.05$) but failed to rescue mice from colitis-induced weight loss (Fig. 1, A–D). As opposed to the intracolonic route, intraperitoneal administration of PAR_1 agonist had no effect on all the inflammatory parameters induced by oxazolone colitis (data not shown). These results indicated that intracolonic but not systemic PAR_1 activation administration inhibited the inflammatory process mediated by oxazolone.

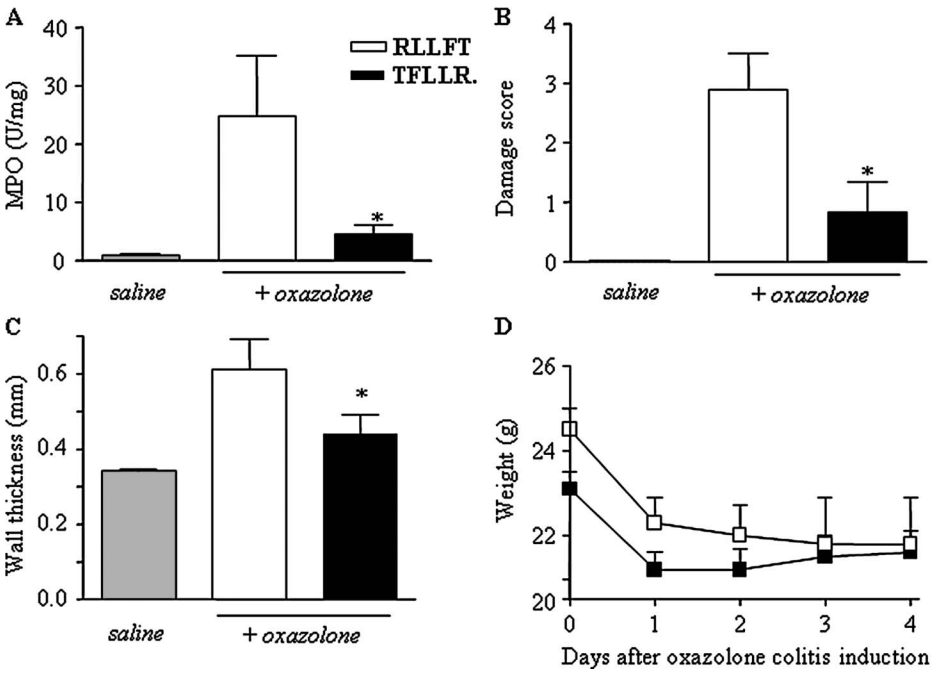

FIGURE 1. Prevention by PAR_1 agonist administration of oxazolone-induced colonic inflammation. Mice treated with oxazolone were pretreated with the PAR_1 agonist, TFLLR, (50 μg/mouse, black bars for A, B, and C and open symbols for D) or by the inverse peptide, RLLFT, (50 μg/mouse, white bars for A, B, and C and closed symbols for D). Inflammatory parameters were (A) MPO activity, (B) damage score, (C) wall thickness, and (D) weight. Values are means ± SEM; n = 8; *, $P < 0.05$ compared with control values.

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Effect of Endogenous PAR$_1$ Activation on the Colonic Inflammation Induced by Oxazolone: Use of PAR$_1$-Deficient Mice (PAR$_1^{-/-}$) and a PAR$_1$ Antagonist

To corroborate an anti-inflammatory role for PAR$_1$ in the setting of oxazolone-induced colitis, we used both PAR$_1$-deficient mice (PAR$_1^{-/-}$) and a PAR$_1$ antagonist. First, we showed that treatment of PAR$_1$-deficient mice by PAR$_1$ agonist had no effect on the colitis mediated by oxazolone as observed by unchanged MPO activity, damage score, and wall thickness (data not shown). In a second series of experiments, we studied whether or not endogenous PAR$_1$ exerts anti-inflammatory effects on the T_H2 cytokine profile colitis mediated by oxazolone. First, we used mice deficient for the *PAR$_1$* gene compared with C57BL wildtype mice. In PAR$_1^{-/-}$ mice, oxazolone-induced increase in macroscopic damage score (4.2 ± 0.6 versus 1.9 ± 0.9; $P < 0.05$), wall thickness (0.88 ± 0.05 versus 0.58 ± 0.05 mm; $P < 0.05$), and MPO activity (120.23 ± 29.96 versus 42.50 ± 15.00 U/mg of proteins; $P < 0.05$) was significantly higher compared with C57BL wildtype mice (Fig. 2, A–C). In PAR$_1$-deficient mice, oxazolone colitis significantly increased weight loss compared with wildtype mice but had no effect on the survival rate (Fig. 2, D and E). In a second set of experiments, we used a PAR$_1$ antagonist and pretreatment in mice injected with oxazolone. PAR$_1$ antagonist treatment, but not vehicle treatment, provoked an increase in oxazolone-mediated inflammatory reaction, characterized by damage score (2.25 ± 0.41

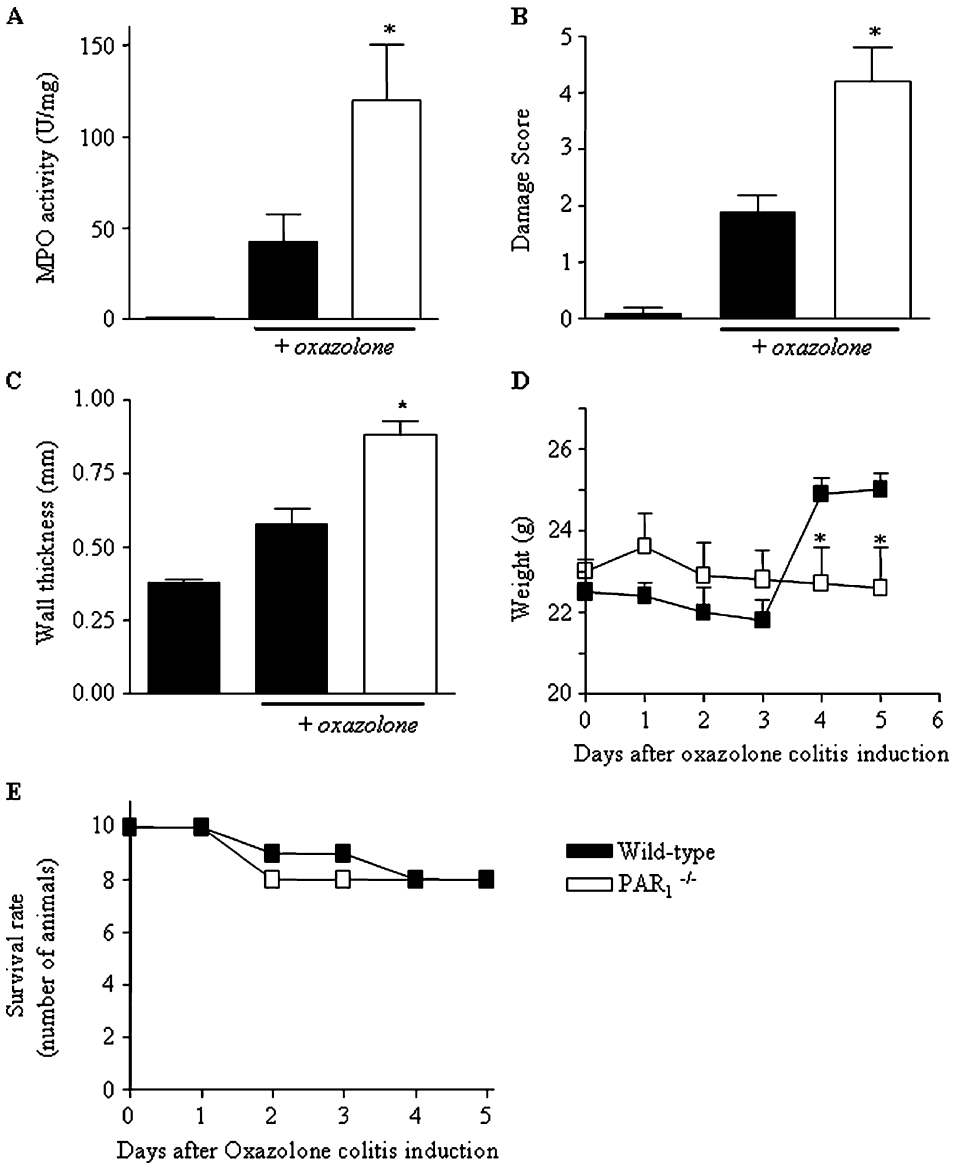

FIGURE 2. The inflammatory reaction mediated by oxazolone was increased in PAR$_1$-deficient mice. Inflammation was observed by (A) MPO, (B) damage score, (C) wall thickness, (D) weight, and (E) survival rate 5 days after oxazolone administration in PAR$_1$-deficient mice (PAR$_1^{-/-}$, white bars and open symbols) and their wildtype littermates (wildtype, black bars and closed symbols). Values are means ± SEM; n = 8 per group. *Significantly different from wild type mice, $P < 0.05$.

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versus 1.14 ± 0.41; $P < 0.05$), MPO activity (53.50 ± 16.40 versus 10.96 ± 2.60 U/mg of protein; $P < 0.05$), and wall thickness (0.66 ± 0.05 versus 0.52 ± 0.03 mm; $P < 0.05$) values significantly higher compared with the ones of mice treated with oxazolone and PAR_1 antagonist vehicle (Fig. 3, A–C). These results indicated that by eliminating the impact of PAR_1 activation either genetically or with a receptor antagonist, the inflammatory reaction caused by oxazolone was increased. This shows that endogenous PAR_1 activation plays a role to reduce the colonic inflammation induced by oxazolone.

Effects of PAR_1-AP Administration on Oxazolone-Mediated Changes in Cytokine Profile

In keeping with our previous observations,[17] PAR_1 activation elicited a T_H1 profile, characterized by a significant increase in IFNγ expression (0.57 ± 0.03 versus 0.11 ± 0.02; $P < 0.05$) and a decrease in IL-4 mRNA expression (0.06 ± 0.01 versus 0.19 ± 0.02; $P < 0.05$) and IL-10 expression (0.04 ± 0.01 versus 0.09 ± 0.01; $P < 0.05$) compared with mice treated with control peptide (Fig. 4, A–C). In contrast, oxazolone treatment induced elevation of T_H2 profile characterized by a significant increase in IL-10 (0.24 ± 0.01 versus 0.091 ± 0.010; $P < 0.05$) and IL-4 (0.52 ± 0.03 versus 0.19 ± 0.02; $P < 0.05$) and a decrease in IFNγ (0.05 ± 0.01 versus 0.11 ± 0.02; $P < 0.05$) mRNA expression compared with control noninflamed mice (Fig. 4, A–C). Moreover, intracolonic treatments with either the PAR_1 agonist or oxazolone increased mRNA expression of 2 inflammatory cytokines, TNFα (0.21 ± 0.05 and 0.27 ± 0.03 versus 0.07 ± 0.01 respectively; $P < 0.05$) and IL-1β (0.22 ± 0.03 and 0.18 ± 0.02 versus 0.03 ± 0.01 respectively; $P < 0.05$) compared with control mice (Fig. 4, D and E). Intracolonic administration of the PAR_1 agonist, TFLLR, prevented oxazolone-induced increases in T_H2 cytokines IL-4 and IL-10, and coadministration of PAR_1-agonist and oxazolone also significantly reduced the increased expression of the proinflammatory cytokines TNFα and IL-1β. In mice treated with both the PAR_1 agonist and oxazolone, mRNA cytokine expression for TNFα, IL-1β, IL-4, IFNγ, and IL-10 was not different from noninflamed mice (Fig. 4, A–E).

DISCUSSION

The most significant finding of our study was that PAR_1 activation, which on its own can cause colonic inflammation,[17] can paradoxically play an anti-inflammatory role in the setting of a T_H2 cytokine-mediated inflammation. Confirming previous reports, this study showed that oxazolone-induced colitis confers a T_H2 cytokine profile. Skin sensitization and the subsequent intracolonic administration of oxazolone provoked an inflammatory reaction characterized by an increase in granulocyte infiltration, tissue damage, and IL-4, IL-10, TNFα, and IL-1β mRNA expression (Figs. 1 and 4). The intracolonic coadministration of a PAR_1 agonist attenuated the different hallmarks of inflammation such as the increase of tissue MPO activity, a marker of granulocyte infiltration, the damage scores, increased wall thickness as a marker of edema formation or hyperplasia, and inflammatory cytokine mRNA expression (Figs. 1 and 4). These anti-inflammatory effects of the PAR_1 agonist are specific to the oxazolone-induced colitis, because in 2 other models of IBD (TNBS and the dextran sodium sulfate (DSS) models), PAR_1 activation exacerbates rather than diminishes the colitis.[17] In contrast with the T_H2 profile exhibited by the oxazolone model, the DSS and the TNBS models are known for exhibiting a T_H1 cytokine profile, and in these models, PAR_1 activation increased the inflammatory reaction. Thus, the inflammatory cytokine milieu would seem to determine whether PAR_1 activation will cause pro- or anti-inflammatory effects. In these 3 inflammatory models, endogenous PAR_1 activation played a role in the development of colitis: inducing inflammation in the TNBS and DSS models and being protective against inflammation induced by oxazolone. Interestingly, in PAR_1-deficient mice or in wildtype mice treated by the PAR_1 antagonist, the inflammatory reaction mediated by oxazolone was exacerbated compared with the inflammation in untreated or vehicle-treated wildtype mice (Figs. 2 and 3). Thus, both a pharmacological and a genetic approach established beyond question that, in the T_H2 model of colitis, it is PAR_1, and not another receptor, that confers protection.

In oxazolone colitis, the T-cell response is an IL-4–driven T_H2 T cell reaction, which is characterized by elevated IL-4/IL-5 production and low IFNγ production. This T-cell response can be prevented by the systemic coadministration of

FIGURE 3. Treatment with PAR_1 antagonist increased oxazolone-induced inflammatory reaction. Effect of systemic treatment with a PAR_1 antagonist (white bars) or its vehicle (black bars) on (A) MPO activity, (B) damage scores, and (C) wall thickness in mice 5 days after induction of colitis by intracolonic administration of oxazolone. Values are means ± SEM; $n = 8$ per group. *Significantly different from control group, $P < 0.05$.

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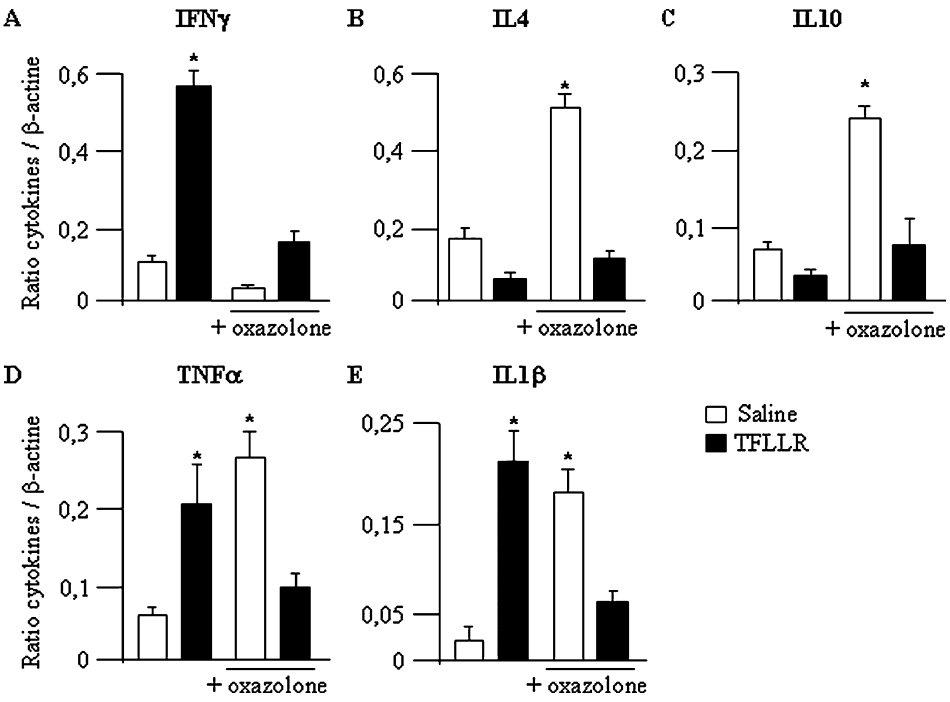

FIGURE 4. Prevention by PAR$_1$ agonist administration of oxazolone-induced increase in inflammatory and T_H2 cytokines mRNA expression. Cytokine mRNA expression in mouse colon after the intracolonic administration of the PAR$_1$ agonist, TFLLR (50 μg/mouse, black bar), oxazolone, or both. Values are mean ± SEM, n = 8 per group. *Significantly different from control group, $P < 0.05$.

anti-IL-4 antibodies.[21] Oxazolone treatment also provokes the recruitment and the proliferation of T_H2 NK-T cells producing IL-13.[22] In that same study, using a histochemical-morphometric analysis, the authors showed that oxazolone colitis mimics many aspects of human ulcerative colitis (UC). It has been shown that human UC is associated with an increase in IL-5 secretion; nevertheless, UC is not a real T_H2 inflammation because it is not associated with an increase in IL-4 secretion, which also characterizes the T_H2 response.[23] However, just as the TNBS colitis is a model considered close to Crohn's disease (CD), it seems reasonable to consider oxazolone as a model close to UC. In a previous study, we showed an increase in PAR$_1$ expression in colonic biopsies from patients with UC and CD.[17] However, in patients with CD, a 2-fold increase in the level of PAR$_1$ expression was observed compared with that in colons of patients with UC.[17] Whereas the overexpression of PAR$_1$ in patients with CD might contribute to the proinflammatory signals associated with a T_H1 cytokine profile, the increase of PAR$_1$ in patients with UC might have opposite effects. From the results of this study, we hypothesized that, in UC, PAR$_1$ could act as a primary signal of defense against the expansion of the inflammation by trying to regulate the T_H1/T_H2 cytokine balance.

In the colon of patients with IBD, PAR$_1$ is expressed on monocytic cells like lymphocytes.[17] Interestingly, the proinflammatory effects of the PAR$_1$ agonist observed in wildtype mice are completely absent in SCID mice or in mice deficient for the ZAP-70 protein, which causes the absence of mature lymphocytes. These results implicate a role for B and T lymphocytes in the ability of PAR$_1$ activation to cause chronic inflammation of the bowel.[17] Moreover, on lymphocytes, PAR$_1$ activation increases tyrosine phosphorylation of Vav1, which is implicated in T-cell receptor (TCR)-mediated effects.[24] In addition to Vav1 and Lck, PAR$_1$ activates SLP-76 and ZAP-70, 2 other key proteins in TCR activation of T cells.[24] Another study has shown that PAR$_1$ activation leads to mitogenesis in T cells, the production of IL-2, and the activation of antigen CD69, which are also induced by TCRs.[25] PAR$_1$ activation in human monocytic cell line U937 increased the secretion of IL-8; this increase was regulated by IFNγ release, confirming the regulation of monocytic cells by PAR$_1$.[26] Taken together these studies suggest that activation of lymphocyte PAR$_1$ may play a crucial role in chronic inflammatory diseases. Our results showed that PAR$_1$ activation increases the secretion of T_H1 cytokines, which are induced by IL-2. Thus, in this model of T_H2 colitis, PAR$_1$ activation can directly activate lymphocytes and increase the number and differentiation of T_H1 lymphocytes coming to the site of inflammation, thereby restoring the balance with T_H2 cytokines and resolving the inflammation. This protective effect would be consistent with the numerous in vivo studies that have shown that T_H1 and T_H2 responses are mutually counter-regulatory.

Given that PAR$_1$ activation may lead to a reduction of T_H2 cytokine-associated inflammation, a major question is what proteinases might lead to the activation of PAR$_1$ in the inflammatory setting? Our results show that endogenous PAR$_1$ activation is implicated in the reduction of inflammation mediated by the oxazolone. Thereby, endogenous PAR$_1$ activating proteases participate in the reduction of the inflammatory reaction. Thrombin has been shown to activate PAR$_1$ in vitro and is considered as the primary endogenous activator of PAR$_1$

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on platelets.[27] Other proteases from the coagulation cascade, such as factor Xa or VIIa, have also been shown to activate PAR_1[28] and would be very good candidates to activate PAR_1 on lymphocytes in the setting of colitis. No study has yet reported the effects of intestinal bacteria (pathogens or nonpathogens) on PAR activation, although PAR_1 can be activated by proteases from pathogens such as the mouth pathogen *P. gingivalis*.[3] Therefore, in the context of UC, because intestinal microflora is known to be implicated in the pathogenesis of IBD, we cannot rule out the possibility that bacterial proteases might be implicated in PAR_1 activation and might participate in the switch of the cytokine balance.

In conclusion, our study shows that PAR_1 activation in the setting of T_H2 colitis resolves the inflammatory reaction. This study supports our hypothesis of a role of PAR_1 in IBD by the regulation of the T-cell population. In contrast to our first study with T_H1 models of IBD, in the case of T_H2 inflammation, PAR_1 activation can have a beneficial effect on the resolution of inflammation. The inflammatory setting in which PAR_1 acts (i.e., T_H1- or T_H2-associated inflammation) becomes a critical factor to consider in terms of therapeutic efficacy. One can therefore anticipate a potential therapeutic role for both PAR_1 agonists and antagonists, depending on the nature of the inflammatory process. Because IBDs are immune-mediated disorders, wherein an imbalance between T_H1 and T_H2 cytokine profiles is thought to play a major pathogenic role, PAR_1 activation or inhibition might appropriately tip the balance to resolve inflammation.

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