Vanin-1 licenses inflammatory mediator production by gut epithelial cells and controls colitis by antagonizing peroxisome proliferator-activated receptor γ activity

Carole Berruyer,1,2,3 Laurent Pouyet,1,2,3 Virginie Millet,1,2,3 Florent M. Martin,1,2,3 Aude LeGoffic,1 Alexandra Canonici,1 Stéphane Garcia,4 Claude Bagnis,5 Philippe Naquet,1,2,3 and Franck Galland1,2,3

1Centre d'Immunologie de Marseille-Luminy, Université de la Méditerranée, 13288 Marseille Cedex 9, France
2Institut National de la Santé et de la Recherche Médicale (INSERM) U631, 13288 Marseille Cedex 9, France
3Centre National de la Recherche Scientifique (CNRS) UMR6102, 13288 Marseille Cedex 9, France
4Service d’Anatomie Pathologique, Faculté de Médecine Nord, 13915 Marseille Cedex 9, France
5Établissement Français du Sang Alpes Méditerranée, 13005 Marseille Cedex 9, France

Inflammation of the gastrointestinal tract that manifests as ulcerative colitis and Crohn’s disease in human. These pathologies exemplify the rupture of equilibrium between bacterial-driven inflammation and tolerogenic gut immunity (1–3). In healthy individuals, the intestinal mucosa is in a state of controlled inflammation and its homeostasis depends upon intimate contacts between innate immune and epithelial cells. Dendritic cells and macrophages participate in the early detection of inflammatory stress and trigger destructive responses associated with colitis. However, because of their frontline position, intestinal epithelial cells (IECs) likely play a key role in stress sensing. They are known to express Toll-like receptors and MHC molecules and to secrete antibacterial peptides and chemokines upon activation. It is therefore important to evaluate the hierarchical contribution of epithelial cells to stress sensing and triggering of gut inflammation.

Vanin-1 is an epithelial ectoenzyme with a pantetheinase activity that provides cysteamine/cystamine to tissue. Using the 2,4,6-trinitrobenzene sulfonic acid (TNBS)-colitis model we show here that Vanin-1 deficiency protects from colitis. This protection is reversible by administration of cysteamine or bisphenol A diglycidyl ether, a peroxisome proliferator-activated receptor (PPAR)γ antagonist. We further demonstrate that Vanin-1, by antagonizing PPARγ, licenses the production of inflammatory mediators by intestinal epithelial cells. We propose that Vanin-1 is an epithelial sensor of stress that exerts a dominant control over innate immune responses in tissue. Thus, the Vanin-1/pantetheinase activity might be a new target for therapeutic intervention in inflammatory bowel disease.

Correspondence
Franck Galland:
fgpn@ciml.univ-mrs.fr

Abbreviations used: ATRA, all-trans-retinoic acid; BADGE, bisphenol A diglycidyl ether; COX, cyclooxygenase; 15-d-PGJ2, 15-deoxy-Δ12,14-prostaglandin J2; GSH, glutathione; IBD, inflammatory bowel disease; IEC, intestinal epithelial cell; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein; PPAR, peroxisome proliferator-activated receptor; SCID, severe combined immunodeficient; TNBS, 2,4,6-trinitrobenzene sulfonic acid.

Colitis involves immune cell–mediated tissue injuries, but the contribution of epithelial cells remains largely unclear. Vanin-1 is an epithelial ectoenzyme with a pantetheinase activity that provides cysteamine/cystamine to tissue. Using the 2,4,6-trinitrobenzene sulfonic acid (TNBS)-colitis model we show here that Vanin-1 deficiency protects from colitis. This protection is reversible by administration of cysteamine or bisphenol A diglycidyl ether, a peroxisome proliferator-activated receptor (PPAR)γ antagonist. We further demonstrate that Vanin-1, by antagonizing PPARγ, licenses the production of inflammatory mediators by intestinal epithelial cells. We propose that Vanin-1 is an epithelial sensor of stress that exerts a dominant control over innate immune responses in tissue. Thus, the Vanin-1/pantetheinase activity might be a new target for therapeutic intervention in inflammatory bowel disease.
stress can be generated by acute exposure to nonsteroidal anti-inflammatory drugs or chronic *Schistosoma mansoni* infection. In both situations, Vanin-1<sup>−/−</sup> mice were shown to control the resulting inflammation and ensuing hemorrhages that normally provoke death (7). Collectively, these observations pointed to a proinflammatory role of Vanin-1, which may be involved in the development of IBDs. These pathologies result from an excessive immune response to gut-derived infectious stimuli in a context of unbalanced immune tolerance. Therefore, we investigated an experimental model of colitis after intrarectal administration of 2,4,6-trinitrobenzene sulfonic acid (TNBS) (8). This model has been widely used to dissect some of the molecules and the cellular mechanisms involved in acute or chronic colitis. Among them, the peroxisome proliferator-activated receptor (PPAR)<sub>γ</sub> was identified as a checkpoint in the control of antiinflammatory responses and gut homeostasis (9, 10). Indeed, PPAR<sub>γ</sub> heterozygous mice exhibit increased susceptibility to experimental TNBS-colitis, and in human, ulcerative colitis patients display an impaired expression of PPAR<sub>γ</sub> in colonic epithelial cells (11, 12). In addition, ligand activation of PPAR<sub>γ</sub> was shown to reduce the severity of colitis in mouse models, and it was recently demonstrated that the positive clinical effect of 5-aminosalicylic acid in the treatment of IBD patients was linked to PPAR<sub>γ</sub> activation (13, 14). PPAR<sub>γ</sub> is a member of the steroid receptor family with various biological functions including differentiation, apoptosis, lipid metabolism, and antiinflammatory properties (15, 16). Ligands for PPAR<sub>γ</sub> include few identified natural compounds like polyunsaturated fatty acids, some eicosanoids, the 15-deoxy-Δ<sup>12,14</sup>-prostaglandin J<sub>2</sub> (15-d-PGJ<sub>2</sub>), and certain drugs like the thiazolidinedione (17–20). After activation, PPAR<sub>γ</sub> forms heterodimer with the retinoic X receptor and the resulting complex binds PPAR-responsive elements (PPREs) within target gene promoters (15). Here we show that Vanin-1 modulates both the expression and the ligand-induced activation of PPAR<sub>γ</sub> in epithelial cells, therefore, regulating the expression of downstream proinflammatory target genes. Control of PPAR<sub>γ</sub> gene expression and function explains why Vanin-1 mutant mice better resist to colitis. These studies further show the importance of Vanin-1–dependent epithelium-derived signals in the triggering of colitis.

**RESULTS**

Vanin-1<sup>−/−</sup> mice resist colitis-causing death: an epithelial-based mechanism

Mice were subjected to TNBS-induced colitis which provoked a rapid weight loss and death of 80% control BALB/c mice within 10 d (Fig. 1, A and B). In contrast, 70% of the Vanin-1<sup>−/−</sup> mice survived during the same period and did not show considerable weight loss. Since Vanin-1 controls the production of cysteamine in vivo, we administered cystamine (120 mg/kg) 2 d before TNBS injection and daily until death or sacrifice. This treatment completely reversed the protection conferred by the lack of Vanin-1 toward colitis since 90% of cystamine–treated Vanin-1<sup>−/−</sup> mice died within 5 d (Fig. 1 A). Thus, cystamine restores the WT–like susceptibility to TNBS-induced colitis in Vanin-1<sup>−/−</sup> mice.
Under normal conditions, Vanin-1 is predominantly expressed by epithelial but not hematopoietic cells. To definitively establish the major contribution of epithelial cells in the control of colitis, we generated chimeras by reconstituting BALB/c control or Vanin-1−/−-irradiated mice with WT or Vanin-1−/− Thy1-depleted bone marrows. These chimeric animals were subsequently submitted to TNBS-induced colitis and their survival was checked over 5 d. As shown in Fig. 1 C, Vanin-1−/− recipients displayed a considerably reduced mortality rate independently of the genotype of the grafted bone marrow. Indeed, ~40–50% Vanin-1−/− versus 0–10% control mice survived. Because the differential sensitivity to colitis might reflect the impact of the Vanin-1 mutation on the development of gut lymphocytes, we introduced the Vanin-1 mutation on the severe combined immunodeficient (SCID) mouse background. As shown in Fig. 1 D, although the lack of lymphocytes was associated with a reduced mortality, Vanin-1-deficient SCID BALB/c mice better resisted TNBS-colitis compared with Vanin-1+/+ SCID controls.

Thus, the protection conferred by the absence of Vanin-1 against lethal TNBS-colitis depends on a primordial epithelial-based response. These results document the preponderant role played by epithelial cells in the control of the severity of the colitis.

Lack of Vanin-1 is associated with mild tissue damage after colitis induction
The colon of mice was examined 2 d after TNBS injection, and the severity of clinical signs of colitis was evaluated macroscopically using the Wallace score. As shown in Fig. 2 A, control mice had severe macroscopic lesions characterized by numerous hemorrhagic and necrotic zones widely distributed along the whole colon and extending into the cecum corresponding to a Wallace score of 8 (Fig. 2 B). In contrast, Vanin-1−/− colons remained relatively healthy (Wallace score ∼ 4).

Histologically, TNBS-treated WT colons were characterized by a severely disrupted mucosa with large areas of ulceration and extensive necrosis sparing few glands, subepithelial edema, and dense inflammatory infiltrate (Fig. 2 C). In contrast, the improved survival of Vanin-1−/− mice was correlated to considerably less alterations of the colon with preserved mucosa integrity, reduced submucosal edema, and poorer leukocyte infiltration. This was quantified on several TNBS-treated animals using the Ameho score and represented in Fig. 2 D. As expected, cystamine-treated Vanin-1−/− mice showed similar alterations to that observed in control animals (Fig. 2). These results demonstrate that Vanin-1 deficiency in the epithelial compartment prevents massive tissue infiltration by inflammatory cells and consequently the development of colonic lesions.

Vanin-1 controls the inflammatory response in injured colon
To further document the impact of Vanin-1 deficiency on colitis we scored the production of inflammatory effectors in colon of mice killed 2 d after TNBS injection. Using a cytokine array (Fig. 3, A and B), we observed the induction of a particular set of cytokines and chemokines in the colons of WT mice compatible with a major inflammatory reaction. This set includes the proinflammatory cytokine IL-6 tissue inhibitor of metalloproteinase-1, involved in tissue remodeling and wound healing, the neutrophil chemoattractant macrophage inflammatory protein (MIP)–2 and keratinocyte chemotactant, and MIP-1α and monocyte chemoattractant...
Vanin-1−/− colons which was further confirmed by flow cytometry analysis of Mac1high-F4/80+ macrophages and Mac1lowGr1+ granulocytes obtained from dissociated tissues (unpublished data).

Bisphenol A diglycidyl ether, a PPARγ antagonist, abrogates Vanin-1−/− protection against colitis

PPARγ activation by agonists exerts antiinflammatory effects and attenuates TNBS-colitis (11, 21). This incited us to determine whether PPARγ contributed to the down-regulation of the inflammatory processes observed in TNBS-treated Vanin-1−/− mice. This was investigated by daily intraperitoneal administration of the bisphenol A diglycidyl ether (BADGE) PPARγ antagonist (30 mg/kg), starting 2 d before TNBS injection (22, 23). All BADGE-treated mice developed a severe colitis to a level comparable to that of TNBS-exposed BALB/c mice. In Vanin-1−/− mice, BADGE administration restored the sensitivity to TNBS-colitis recapitulating typical colon lesions with high inflammatory cell infiltrates and necrotic lesions (Fig. 4, A–C). This was not caused by an intrinsic toxicity of BADGE itself because mice submitted to an injection of BADGE alone, without intrarectal TNBS administration, did not die or present sign of colonic damage over the 15 d of experimentation (unpublished data). These findings suggested that the protective phenotype associated with the Vanin-1 deficiency was fully abrogated by PPARγ inactivation.

Vanin-1 regulates the expression of PPARγ in epithelial cells

Because BADGE suppresses the resistance of the Vanin-1−/− mice to TNBS-induced inflammation, we explored whether the Vanin-1 molecule directly regulated PPARγ expression and/or activation. First, despite high variability between animals we did not find a considerable difference in PPARγ protein expression between control and Vanin-1−/− mice by Western blot analysis on whole colon tissue extracts (Fig. 4 D). After TNBS administration, a comparable increase in PPARγ expression was observed in WT and Vanin-1−/− colons (Fig. 4 D). In Vanin-1−/− mice, cystamine administration, which abrogates protection against colitis only slightly, reduced PPARγ levels, whereas BADGE administration had no impact. Thus, on whole colon extracts, PPARγ expression did not correlate with the clinical outcome. Furthermore, BADGE treatment, despite its antagonistic effect, did not seem to modulate PPARγ expression. A major difficulty in this type of analysis is caused by the largely distributed PPARγ expression among numerous cell types in colon, whereas Vanin-1 expression is mostly restricted to epithelial cells. Therefore, we performed the same analysis on protein lysates obtained from purified resting IECs, which showed considerably higher PPARγ expression levels in Vanin-1−/− compared with control IECs (Fig. 4 E). Because of the toxicity of TNBS administration, we were unable to obtain enough viable purified IECs from TNBS-treated mice to be able to perform the same analysis.

Figure 3. Vanin-1 controls the inflammatory response in injured colon. (A) ELISA-based cytokine arrays were hybridized with total colon tissue lysates derived from control or Vanin-1−/− mice 2 d after colitis induction. Relevant signals are indicated. (B) Quantification of signal intensities resulting from the cytokine array experiment. (C) Comparative Western blot analysis of the expression of COX-2 in control and Vanin-1−/− mice before and 2 d after TNBS administration. Quantification of the results is represented in the bottom panel. (D) Similar experiment including total colon extracts from Vanin-1−/− mice submitted to a treatment with cystamine. Quantification is shown in the bottom panel.
We then turned to similar analyses on two distinct cell lines. The mouse MTE4.14 thymic epithelial cell line naturally expresses Vanin-1. Using a lentiviral-based RNA interference strategy, we derived clones showing an 80% reduction of Vanin-1 cell surface expression (named Vanin-1RNAi MTE4.14 cells). In parallel, the immature mouse intestinal cell line mIC, which lacks Vanin-1 expression, was transfected with Vanin-1 cDNA to generate stable Vanin-1(+)(mIC) transfectants. As shown in Fig. 5, the absence or reduced level of Vanin-1 was systematically associated with an up-regulation of the PPARγ mRNA, quantified by real-time PCR. Indeed, levels of PPARγ mRNA were, respectively eight- and fivefold higher in the Vanin-1RNAi MTE4.14 and the Vanin-1(−) mIC cells than in their Vanin-1(+) counterparts (Fig. 5 A). This was correlated with changes in PPARγ protein level and measured by Western blot analysis (Fig. 5 B). The augmented PPARγ mRNA expression could be reversed by incubating Vanin-1RNAi MTE4.14 cells with 1 nM cystamine for 12 h (Fig. 5 C). The prostaglandine 15-d-PGJ2 has been identified as a potent endogenous ligand of PPARγ that also increases PPARγ mRNA expression in cell lines (24, 25). We found using real-time PCR that 15-d-PGJ2 (5 μM) boosted PPARγ mRNA expression in Vanin-1(−) MTE4.14 cells by sixfold but did not further augment that found in Vanin-1RNAi MTE4.14 where the level of PPARγ expression was already high (Fig. 5 D). In conclusion, we document a reverse relationship between Vanin-1 and PPARγ transcript levels and an impact of cystamine on PPARγ transcription.

The activation of PPARγ is modulated by Vanin-1 in epithelial cells

Activation of PPARγ results in the translocation of the protein into the nucleus and its subsequent binding to specific DNA elements called PPREs (15). We investigated whether Vanin-1 could modulate the degree of PPARγ activation by analyzing the intracellular distribution of a GFP-tagged PPARγ molecule transfected in the mIC cells. In unstimulated cells, the localization of the fluorescent tag was predominantly cytoplasmic. Exposure of cells to 15-d-PGJ2 (5 μM) for 12 h provoked a considerable nuclear translocation of the GFP-tagged PPARγ in Vanin-1(−) but less so in Vanin-1(+) mIC cells (Fig. 6 A). To quantify this parameter, we investigated PPARγ transcriptional activity by transient transfection of a 3XPPRE-luciferase reporter construct in MTE4.14 cells. As already reported in other cellular models (26, 27), 15-d-PGJ2 induced a threelfold increase of the reporter-gene activity in Vanin-1(−) cells. In contrast, this treatment had no detectable effect in Vanin-1(+) mIC cells (Fig. 6 B). To quantify this parameter, we investigated PPARγ transcriptional activity by transient transfection of a 3XPPRE-luciferase reporter construct in MTE4.14 cells. As already reported in other cellular models (26, 27), 15-d-PGJ2 induced a threelfold increase of the reporter-gene activity in Vanin-1(−) cells. In contrast, this treatment had no detectable effect in Vanin-1(+) mIC cells (Fig. 6 B). Given the modest level of PPRE-mediated transcription in control cells, we combined PPARγ ligands with a retinoic acid receptor agonist (i.e., all trans-retinoic acid [ATRA]) previously shown to enhance the PPARγ-dependent effects and to induce maturation of myelomonocytic cells (28). In MTE4.14 epithelial cells, this combination considerably amplified the detectable reporter-gene activity in the Vanin-1(−) cells. In contrast, in Vanin-1(+) cells the reporter gene activity was still barely detectable (Fig. 6 B). This shows that the presence of the Vanin-1 molecule prevents the activation of PPARγ in response to agonists.

Figure 4. BADGE abrogates the protection against colitis associated with Vanin-1 deficiency. (A) Comparative survival rates of untreated and BADGE-treated control and Vanin-1−/− mice during TNBS-colitis. (B) Macroscopic appearance of colons from BADGE-treated control and Vanin-1−/− mice 2 d after TNBS induction. (C) Representative histological colon sections 2 d after TNBS administration. (D) Western blot analysis of the PPARγ expression in total colon extracts from control, Vanin-1−/− mice, and Vanin-1−/− animals treated with cystamine or BADGE. Samples were prepared 2 d after TNBS induction. The bottom panel represents a quantification of the results. (E) Western blot analysis of the expression of the PPARγ protein by IEC purified from control or Vanin-1−/− colons. Quantification is shown in the bottom panel.
Vanin-1 regulates IL-1β-driven chemokine release by epithelial cells via modulation of PPARγ activation

To demonstrate that Vanin-1 acts autonomously at the level of epithelial cells, we extended in vitro our analysis. We then showed by real-time quantitative PCR analysis that IL-1β (10 ng/ml), a potent proinflammatory stimulus, induced the production of MCP-1 and MIP-2 in the Vanin-1(+) MT4.14 or mIC cells, but not in their Vanin-1(−) counterparts (Fig. 7). A similar result was observed with COX-2 in mIC cells (unpublished data). Therefore, Vanin-1 expression licenses the epithelial cell response to a proinflammatory stimulus through the specific transcription of target genes.

To demonstrate further that PPARγ is a main target of Vanin-1 proinflammatory activity, we evaluated the effect of PPARγ agonists and antagonists on the production of the MCP-1 or MIP-2 chemokines by Vanin-1(+) or (−) cells (Fig. 7). Stimulation of the Vanin-1(+) cell lines (MTE4.14 and mIC transfectants) by IL-1β (10 ng/ml for 12 h) resulted in the transcription of MCP-1 or MIP-2 mRNAs (approximately threefold increase), which was abrogated by the simultaneous incubation with the PPARγ agonist 15-d-PGJ2 (5 μM). Interestingly, the deficiency in chemokine production by IL-1β-stimulated Vanin-1(−) cells (RNAi MTE4.14 or mIC) was almost fully restored by antagonizing PPARγ activation with BADGE (5 μM). Hence, modulation of PPARγ activation by agonists and antagonists influenced the
Vanin-1−/−: a novel mechanism of colonic protection against oxidative stress.

In healthy individuals, the gut buffers the constant low level of environmental stress originating from the diet and the conventional microflora. A normal epithelium provides an effective barrier against luminal agents. When the integrity of the barrier is compromised, however, innate mechanisms of defense are triggered to eliminate the danger, repair the tissue, and restore a homeostatic state. In this study, we have addressed the role of epithelial Vanin-1 in the development of colitis and show that Vanin-1 deficiency reduces colitis severity in the TNBS model. This is clearly demonstrated by an improved survival of Vanin-1−/− mice compared with WT animals after TNBS treatment. Furthermore, treatment of these mice by a PPARγ antagonist (BADGE) abrogates their protection against TNBS-colitis. Thus, Vanin-1 appears to control TNBS-colitis by antagonizing PPARγ activity.

Vanin-1 regulates oxidative stress in the gut

Vanin-1 is an epithelial ectoenzyme that provides cysteamine to tissue through the hydrolysis of pantetheine, a vitamin B5 intermediate. In mice, Vanin-1 deficiency confers resistance to systemic oxidative stress associated with ionizing radiations or exposure to xenobiotics (6). Thus, Vanin-1 participates in the response to stress in vivo. In gut, Vanin-1 is expressed by epithelial but not hematopoietic cells. We previously reported that Vanin-1−/− gut shows increased levels of endogenous GSH and that Vanin-1−/− deficient mice are protected against acute intestinal damages induced by oxidative stress associated with Schistosoma mansoni infection or indomethacin administration (7).

In the murine model of colitis, TNBS administration engenders oxidative stress through its metabolism by depleting GSH and generating reactive oxygen species (29). Overproduction of reactive oxygen species by immune cells contributes to tissue injuries observed in colitis, and a mucosal GSH deficiency is observed in IBD (30–32). This deficit is mainly caused by the decreased activities of key enzymes in GSH synthesis like γ-glutamylcysteine synthetase and γ-glutamyltransferase (32, 33). In contrast, antioxidant therapy with N-acetylcysteine, a GSH precursor, attenuates the acute colitis through increased mucosal GSH levels (33, 34). Thus, impaired mucosal antioxidant capacity may further promote oxidative damage, whereas increased mucosal antioxidant activity prevents oxidative damage. This might explain the better survival and the mild inflammatory response of Vanin-1−/− mice after induction of colitis by TNBS.

Vanin-1 regulates epithelial cell-driven inflammation

Invasion of the mucosa by leukocytes is a characteristic of the gut inflammation. The role of the IECs in orchestrating this recruitment is however not clearly defined. In vitro, epithelial cell lines respond to proinflammatory signals by secreting cytokines and chemokines. In vivo, specific alteration of the intestinal epithelium was shown to lead to intestinal inflammation (35, 36) and the functional role of epithelium-secreted chemokines was demonstrated by targeting overexpression of MIP-2 in IECs using a transgenic model (37).

We report herein that the absence of Vanin-1 results in a dramatic decrease in the expression of a wide panel of inflammatory cytokines in the colon after TNBS. Consequently, Vanin-1−/− mice exhibit attenuated myeloid cell recruitment and lowered subsequent tissue damage. Using bone marrow–reconstituted chimeras, we definitively show that the decreased susceptibility of Vanin-1−/− mice to experimentally induced colitis was determined at the epithelial level. These findings indicate that IECs may actively alter mucosal immune response.

The TNBS-colitis model is usually considered as a Th1-driven inflammatory model recapitulating clinical aspects of the human Crohn’s disease. However, several arguments highlight the importance of innate immunity in the development of colitis: (a) in human IBD as in mouse experimental models, recruitment and activation of macrophages is a constant feature of the immunopathology; (b) mice devoid of Stat3 specifically in macrophages and neutrophils develop chronic enterocolitis (38); (c) murine colitis is dependent on...
Vanin-1 regulates PPARγ in epithelial cells

PPARγ is a nuclear receptor activated by fatty acid ligands and involved in the control of the cellular response to metabolic and nutritional signals (16). A broad recent study about PPARγ describes a key role of this nuclear factor in the maintenance of mucosal integrity in the intestine and a powerful intestinal antiinflammatory effect (10, 41). PPARγ agonists decrease the severity of colitis (9, 11). Thus, modulation of PPARγ has become of great interest for therapeutic strategies in human IBD (42). PPARγ is expressed by several cell types in the colon, including hematopoietic cells like macrophages or lymphocytes and epithelial cells. The antiinflammatory function of PPARγ is attributed to a transcriptional attenuation of the expression of cytokines, chemokines, or COX-2 by these cells (9). Recently, it has been reported that targeted inactivation of PPARγ in colonic epithelial cells abrogates its protective role in colitis (43), but the regulation of PPARγ in epithelial cells remains poorly understood.

One of our main results is that in vivo a functional PPARγ antagonist (BADGE) abrogates the protective phenotype of the Vanin-1−/− mice against colitis. This clearly positions PPARγ downstream of a Vanin-1 effect. We next demonstrated that in vivo the expression of PPARγ in IECs is modulated by the presence of Vanin-1 or the administration of cysteamine. This was confirmed in vitro using in parallel cellular models based on transfectants and RNA interference. The absence of Vanin-1 is correlated with an increase in PPARγ expression, whereas cysteamine down-regulates its expression. In addition, our results indicate that Vanin-1 directly affects PPARγ activation. As shown using PPRE reporter assays, the activation of PPARγ by its natural agonist ligand 15-d-PGJ2 was improved in Vanin-1(−) epithelial cells compared with Vanin-1(+) counterparts, both at the level of nuclear translocation and transcriptional activity. On the other hand, we showed that Vanin-1(+) epithelial cells significantly respond to IL-1β proinflammatory stimulation by expressing inflammatory genes including MCP-1, MIP-2, or COX-2. In addition, we showed that 15-d-PGJ2 considerably antagonized the effect of IL-1β by inhibiting the expression of these target genes in cells. A similar result was described for pancreatic stellate cells in which PPARγ activation by 15-d-PGJ2 results in the inhibition of inducible MCP-1 expression in IL-1β–stimulated cells (27). Of interest, Vanin-1(−) cells do not respond to IL-1β stimulation, but in contrast, exposure of these cells to BADGE restored the cellular capacity to respond to the IL-1β proinflammatory signal.

Conclusion

Collectively, these findings argue for a Vanin-1–dependent regulation of the function of PPARγ in epithelial cells. This might account for the protective phenotype of Vanin-1–deficient mice against colitis. The absence of Vanin-1 and the consequently decreased levels of cysteamine in tissue will promote PPARγ antiinflammatory effects by inhibition of the transcription of epithelial signals intended to alert the innate effectors of the mucosal immune system. Our data support the idea that the presence of Vanin-1 at the epithelial level is determinant for the perception of stress by innate immune cells. Thus, targeting inflammatory pathways in epithelial cells might help to control the development of colitis. Finally, the manipulation the Vanin-1/pantetheinase activity represents an attractive therapeutic strategy for the treatment of IBD.

MATERIAL AND METHODS

Mice. Mice were kept in a specific pathogen-free mouse facility and handled according to the rules of Décret no. 87–848 du 19/10/1987, Paris. All experiments were performed on 6–8-wk-old Vanin-1−/− mice or WT BALB/c littermate controls. Vanin-1−/− lymphocyte-deficient mice used for experiments were generated by introducing the Vanin-1 deficiency on a BALB/c SCID background. To generate chimeras, Vanin-1−/− and BALB/c mice were total body irradiated (10 Gy) before receiving 107 T cell–depleted bone marrow cells obtained from Vanin-1−/− or BALB/c donors.

TNBS-colitis. Acute colitis was induced by an intrarectal administration of 100 μl containing 3 mg of TNBS (Sigma-Aldrich) in 30% ethanol. Cysteamine (Sigma–Aldrich) or BADGE (Sigma–Aldrich) treatments were performed by a daily intraperitoneal administration of a 120 or 30 mg/kg dose, respectively, starting 2 d before TNBS treatment. Acute colitis was assessed 2 d after infusion of TNBS. Colonos were examined by two independent individuals to evaluate the macroscopic lesions according to the Wallace criteria (44). Colon tissue samples located precisely 2 cm above the anal canal were prepared for histological staining with hematoxylin and eosin. Histologic grades were assigned in a blind manner by one pathologist according to the Ameho criteria (45).

Cytokine antibody array. Mouse cytokine antibody array membranes (RayBiotech) were incubated overnight at 4°C with 5/0 μg of total protein lysates prepared from colon tissues according to the manufacturer’s instructions. Detection of signal intensities was performed using a chemiluminescence imaging system (Intelligent Dark Box II; FUJIFILM). Relative cytokine expression levels were determined using the Multi Gauge V2.3 software (FUJIFILM). Internal controls included in the array were used to normalize the results from different membranes. Experiments were performed in duplicate for each mouse genotype.

Mouse epithelial cell lines. MTE4.14 and mIC cells are Vanin-1–expressing mouse thymic epithelial and non-Vinan-1–expressing IECs, respectively. We used a lentiviral-based strategy to generate both Vanin-1(−) mIC cells and MTE4.14–derived stable clones with ~ 80% reduction of the cell
surface expression of Vanin-1 by means of the RNA interference technology (named herein MTE4.14-Vann-1 RNAs). In brief, full-length Vann-1 coding sequences were inserted into pRRLpgkEGFPp518 (provided by D. Trono, University of Geneva, CH-1211, Geneva, Switzerland) by replacing the enhanced green fluorescent protein (EGFP) gene (pRRLpgkVanin1) (46). In parallel, Vann-1 oligonucleotide encoding for shRNA was selected following the criteria described by Rubinson et al. (46) (5′-TGGGTAT-TCTAGCACCCGTACCTAGGCATGATACCATT-TTTTCTC-3′ combined with the following complementary strand 3′-ACACCATAGACCTGGCCTTCTCTGACCTATCTAATGG-TGAAAAAGACT-5′) and placed under the control of the U6 promoter insertion into the Lentilox 3.7 vector (LL3.7; provided by Luke van Parijs, Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, MA) to generate LL3.7shRNAVanin1. Lentiviral particle stocks were produced according to a previously described protocol (46). Viral titers were assessed by quantifying the percentage of EGFP or Vann-1 (+) HUH7 target cells by flow cytometry (FacsCalibur; Becton Dickinson). Membrane expression of Vann-1 was assayed using the 407 monoclonal antibody (4). The lentiviral preparations showed viral titers ranging from 2 to 5 × 10^6 IU/ml. No replication competent virus was detected in any of viral stocks. MTE4.14 cells were transduced with LL3.7shRNAvanin1 or LL3.7 vector as control in a final volume of 500 μl of 8 μg/ml polybrene with a multiplicity of infection of 40. EGFP expression was used to sort transduced cell populations. We obtained one MTE4.14-Vanin-1RNAi clone showing a stable ~80% reduction of the cell surface expression of Vann-1. In parallel, mIC cells were transduced with pRRLpgkVanin1 or pRRLpgkEGFP as control using a multiplicity of infection of 100.

**Real-time quantitative PCR.** Total RNAs were prepared from tissue samples or harvested cell cultures using the TRIzol reagent (Invitrogen). Reverse transcription was performed using the QuantiTect Reverse Transcription kit (Qiagen). Expression of mouse MCP-1, MIP-2, COX-2, and PPARγ mRNA was measured by real-time quantitative RT-PCR method using SYBR green Master Mix (ABI) and the ABI PRISM 7700 sequence detection system (PE Applied Biosystems). Relative mRNA levels were determined by the comparative threshold cycle (2^-ΔΔCt) method (47). The expression of all mRNAs was normalized to that of actin mRNA. All data shown here result from at least three independent experiments. Sequences of all primers used in this study are the following: MCP-1, forward 5′-ctccagctgtctactcactt-3′ and reverse 5′-gttcagggctgggttaaa-3′, MIP-2, forward 5′-acaagcgggtggtctc-3′ and reverse 5′-gactagtggtcagcatcag-3′, COX-2, forward 5′-tgaagggctgtaggctgtgc-3′ and reverse 5′-tctcactggtctctcgc-3′, and PPARγ, forward 5′-gtagaagggcctgcctcag-3′ and reverse 5′-tgaagggctgtcgtctcgc-3′.

**Western blot analysis.** 30 μg of total protein lysates derived from either harvested epithelial cell cultures, colonic tissue samples, or purified IECs were loaded on SDS-PAGE gels. Membranes were probed overnight at 4°C with antibodies against PPARγ (Santa Cruz Biotechnology, Inc.), COX-2 (Cayman), or actin (Sigma-Aldrich) antibodies followed by the appropriate species-specific horseradish peroxidase conjugate (Sigma-Aldrich) and developed using the enhanced chemiluminescence detection system (GE Healthcare). Quantification of band intensities was performed using the Multi Gauge V2.3 software (FUJIFILM).

**Isolation of IECs.** Colons were cut open longitudinally, and feces was removed by washing with PBS. Colons were then cut in small pieces (2 mm) and carefully rocked in RPMI 10% FBS solution for 10 min at 25°C. Colon pieces were then successively incubated under shaking in collagenase mix (RPMI containing 0.2 mg/ml collagenase, 20 mM Hepes, 2% FCS) for 15 min at 30°C, collagenase mix supplemented with 0.2 mg/ml of dispase plus 25 μg/ml DNase I for 25 min at 30°C, and finally trypsin mix (PBS containing 0.05% trypsin, 0.5 mM EDTA, 0.3% BSA, 50 μg/ml DNase I) for 25 min at 36°C. Resulting preparations were filtered on cell strainers (BD Falcon) and centrifuged. Pelleted cells were then resuspended, and IECs were isolated from lymphoid cells using a gradient of Percoll. Cells were counted and checked for viability.

**Luciferase reporter assay.** The 3XPRE-TK-luc plasmid (48) was provided by ADDGENE. MTE4.14 control and MTE4.14 Vanin-1RNAs clones were cultured in 24-well plates and cotransfected with 250 ng of 3XPRE-TK-luc plus 50 ng of pRL-TK-RN control vector (Promega) using the lipofectamine 2000 (Invitrogen). 24 h later, cells were treated for 12 h with 10 μM 15-ΔPGJ2 (Sigma-Aldrich) alone or in combination with 10 μM of ATRA (Sigma-Aldrich). After treatment, cells were lysed in PLB buffer, and detection of the luciferase transcription was performed using the Dual luciferase assay kit (Promega).

**PPARY translocation.** Vanin-1(+) and (−) mIC cells were seeded on coverslips in 24-well plates at a density of 2 × 10^5 cells/well. After 24 h, cells were transfected with 1 μg of GFP-tagged PPARγ plasmid (provided by J.P. Desreumaux, Institut National de la Santé et de Recherche Médicale U795, Lille, France) using lipofectamine 2000 (Invitrogen). 24 h after transfection, cells were treated with 20 μM of 15-ΔPGJ2 (Sigma-Aldrich) and then PBS washed and fixed in 4% paraformaldehyde. The nuclei were stained with DAPI (Sigma-Aldrich) after permeabilization with 0.1% Triton X-100. Coverslips were mounted with nusoil and visualized with a Zeiss Axiovert fluorescence microscope (Carl Zeiss MicroImaging, Inc.).

**Statistical analysis.** Data are expressed as means ± SD. Values from experimental and control groups were compared using the Student’s t-test. Probability values of P < 0.05 were considered statistically significant.

We thank J. Ewbank and L. Alexopoulou for reading the manuscript. We also thank J.P. Desreumaux for providing the PPARγ-GFP plasmid.

This study was supported by institutional grants from INSERM and CNRS and the Euro Thymaide (no. LSBB-CT-2003-503410) and charitable funds from the Association F. Aupetit, C. Berruyer and F.M. Martin were recipients of a grant from the Ministère de l’Éducation Nationale, de la Recherche et de la Technologie.

L. Pouyet was supported by grants from Bourse Régionale de Recherche Provence Alpes Côte d’Azur and Fondation pour la Recherche Médicale.

The authors have no conflicting financial interests.

Submitted: 2 August 2006
Accepted: 18 October 2006

**REFERENCES**

1. Blumberg, R.S., and W. Strober. 2001. Prospects for research in inflammatory bowel disease. JAMA. 285:643–647.

2. Podolsky, D.K. 2002. Inflammatory bowel disease. N. Engl. J. Med. 347:417–429.

3. Strober, W., I.J. Fuss, and R.S. Blumberg. 2002. The immunology of mucosal models of inflammation. Annu. Rev. Immunol. 20:495–549.

4. Arrand-Lions, M., F. Galland, H. Bazin, V. Zakharyev, B.A. Imhof, and P. Naquet. 1996. Vann-1, a novel GPI-linked perivascular molecule involved in thymus homing. Immunity. 5:391–405.

5. Pitari, G., F. Malergue, F. Martin, J.M. Philipppe, M.T. Masucci, C. Chabret, B. Maras, S. Dupre, P. Naquet, and F. Galland. 2000. Pantetheinase activity of membrane-bound vanin-1: lack of free cysteine in tissues of vanin-1 deficient mice. FEBS Lett. 483:149–154.

6. Berruyer, C., F.M. Martin, R. Castellano, A. Macone, F. Malergue, S. Garrido-Urbani, V. Millet, J. Imbert, S. Dupre, G. Pitari, et al. 2004. Vann-1(+)/− mice exhibit a glutathione-mediated tissue resistance to oxidative stress. Mol. Cell. Biol. 24:7214–7224.

7. Martin, F., M.F. Penet, F. Malergue, H. Lepidi, A. Dessein, F. Galland, M. de Reggi, P. Naquet, and B. Gharib. 2004. Vann-1(+)/− mice show decreased NSAID- and Schistosoma-induced intestinal inflammation associated with higher glutathione stores. J. Clin. Invest. 113:591–597.

8. Neurath, M., I. Fuss, and W. Strober. 2000. TNBS-colitis. Int. Rev. Immunol. 19:51–62.
24. Haslmayer, P., T. Thalhammer, W. Jager, S. Aust, G. Steiner, C. Cuzzocrea, S., B. Pisano, L. Dugo, A. Ianaro, P. Maffa, N.S. Patel, R. Di Paola, A. Ialenti, T. Genovese, P.K. Chatterjee, et al. 2004. Peroxisome proliferator-activated receptor gamma and retinoid X receptor heterodimer in hepatogastroenterological diseases. *Lancet*. 360:1410–1418.

10. Dror, S., G.D. Girmun, L. Tou, J.D. Szwaya, E. Mueller, K. Xia, R.A. Shvidranski, and B.M. Spiegelman. 2005. Hic-5 regulates an epithelial program mediated by PPAR-gamma. *Dev. Cell*. 9:362–375.

23. Cuzzocrea, S., B. Pisano, L. Dugo, A. Ianaro, P. Maffa, N.S. Patel, R. Di Paola, A. Ialenti, T. Genovese, P.K. Chatterjee, et al. 2004. Peroxisome proliferator-activated receptor gamma and retinoid X receptor heterodimer in hepatogastroenterological diseases. *Lancet*. 360:1410–1418.

15. Daynes, R.A., and D.C. Jones. 2002. Emerging roles of PPARs in inflammation and immunity. *Nat. Rev. Immunol*. 2:748–759.

20. Spiegelman, B.M. 1998. PPAR-gamma: adipogenic regulator and thiamine diphosphate kinase gamma ligand 15-deoxy-Delta12,14-prostaglandin J2 induces epithelial inflammatory response. *J. Biol. Chem*. 273:141–147.

28. Konopleva, M., E. Eltner, T.J. McQueen, T. Tsao, A. Sudarikov, W. Hu, W.D. Schober, R.Y. Wang, D. Chiu, S.M. Kornblau, et al. 2004. Peroxisome proliferator-activated receptor gamma and retinoid X receptor ligands are potent inducers of differentiation and apoptosis in leukemia. *Mol. Cancer Ther*. 3:1249–1262.

26. Farrow, B., and B.M. Evers. 2003. Activation of PPARgamma increases PTEN expression in pancreatic cancer cells. *Biochem. Biophys. Res. Commun*. 301:50–53.

27. Masamune, A., K. Kikuta, M. Satoh, Y. Sakai, A. Satoh, and T. Shimosegawa. 2002. Ligands of peroxisome proliferator-activated receptor-gamma block activation of pancreatic stellate cells. *J. Biol. Chem*. 277:141–147.

21. 9: Dubuquoy, L., S. Nutten, S. Pettersson, J. Auwerx, and P. Desreumaux. 2003. Impaired expression of peroxisome proliferator-activated receptor gamma in ulcerative colitis. *Gastroenterology*. 124:1265–1276.

14. Su, C.G., X. Wen, S.T. Bailey, W. Jiang, S.M. Rangwala, S.A. Ensinger, and P. Obrist. 2002. The peroxisome proliferator-activated receptor gamma (PPAR gamma). *Cancer Res*. 62:12953–12956.

11. Desreumaux, P., L. Dubuquoy, S. Nutten, M. Pechumai, W. Englaro, K. Schoonjans, B. Denjard, B. Desvergne, W. Wahli, P. Chambon, et al. 2001. Attenuation of colon inflammation through activators of the retinoid X receptor (RXR)-peroxisome proliferator-activated receptor gamma (PPAR-gamma) heterodimer. A basis for new therapeutic strategies. *J. Exp. Med*. 193:827–838.

31. Kruidenier, L., I. Kuiper, C.B. Lamers, and H.W. Verspaget. 2003. Intestinal oxidative damage in inflammatory bowel disease: semi-quantification, localization, and association with mucosal antioxidants. *J. Pathol*. 201:28–36.

13. Forman, B.M., P. Tontonoz, J. Chen, R.P. Brun, B.M. Spiegelman, and R.M. Evans. 1995. 15-Deoxy-delta 12, 14-prostaglandin J2 is a high affinity ligand for peroxisome proliferator-activated receptor gamma. *J. Biol. Chem*. 270:12953–12956.
45. Ameho, C.K., A.A. Adjei, E.K. Harrison, K. Takeshita, T. Morioka, Y. Arakaki, E. Ito, I. Suzuki, A.D. Kulkarni, A. Kawajiri, and S. Yamamoto. 1997. Prophylactic effect of dietary glutamine supplementation on interleukin 8 and tumour necrosis factor alpha production in trinitrobenzene sulphonic acid induced colitis. Gut. 41:487–493.

46. Rubinson, D.A., C.P. Dillon, A.V. Kwiatkowski, C. Sievers, L. Yang, J. Kopinja, D.L. Rooney, M.M. Ihrig, M.T. McManus, F.B. Gertler, et al. 2003. A lentivirus-based system to functionally silence genes in primary mammalian cells, stem cells and transgenic mice by RNA interference. Nat. Genet. 33:401–406.

47. Livak, K.J., and T.D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(−ΔΔC(T)) Method. Methods. 25:402–408.

48. Kim, J.B., H.M. Wright, M. Wright, and B.M. Spiegelman. 1998. ADD1/SREBP1 activates PPARgamma through the production of endogenous ligand. Proc. Natl. Acad. Sci. USA. 95:4333–4337.