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Transgenic Expression of Vitamin D Receptor in Gut Epithelial Cells Ameliorates Spontaneous Colitis Caused by Interleukin-10 Deficiency

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Abstract

Background—Vitamin D-deficiency is common in patients with inflammatory bowel diseases. The vitamin D receptor (VDR) is a nuclear hormone receptor mediating the activity of vitamin D hormone. Our previous studies showed that intestinal epithelial VDR signaling inhibits colitis by protecting the mucosal epithelial barrier, and this activity is independent of non-epithelial immune VDR actions. Interleukin (IL)-10-deficient mouse is a chronic colitis model that develops colitis due to aberrant immune responses. Here we used IL-10 null (IL-10KO) model to assess the anti-colitic activity of epithelial VDR in the setting of an aberrant immune system.

Methods—We crossed IL-10KO mice with villin promoter-driven human (h) VDR transgenic (Tg) mice to generate IL-10KO mice that carry the hVDR transgene in intestinal epithelial cells (IL-10KO/Tg). IL-10KO and IL-10KO/Tg littermates were studied in parallel and followed for up to 25 weeks.

Results—By 25 weeks of age, accumulatively 79% IL-10KO mice developed prolapse, whereas only 40% IL-10KO/Tg mice did so ($P < 0.001$). Compared to IL-10KO mice, IL-10KO/Tg littermates showed markedly reduced mucosal inflammation in both small and large intestines, manifested by attenuation in immune cell infiltration and histological damage and a marked decrease in pro-inflammatory cytokine production. IL-10KO/Tg mice also showed reduced intestinal epithelial cell apoptosis as a result of diminished PUMA induction and caspase 3 activation.

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Conclusion—These observations demonstrate that targeting hVDR expression to intestinal epithelial cells is sufficient to attenuate spontaneous colitis caused by an ill regulated immune system, confirming a critical role of the epithelial VDR signaling in blocking colitis development.

Keywords

Vitamin D receptor; Interleukin-10; Colitis; Inflammatory bowel diseases; Mucosal epithelial barrier

Introduction

The mucosal barrier of the intestine plays an essential role in preventing mucosal inflammation because it separates luminal bacteria, toxins and antigens from the body. Dysfunctional mucosal barrier or increased mucosal barrier permeability has been associated with inflammatory bowel diseases (IBD) in humans and in experimental colitis models [1,2,3]. Intestinal epithelial cells (IECs) are key components of the barrier that express a high level of vitamin D receptor, a nuclear hormone receptor that mediates the biological activity of the vitamin D hormone, 1,25-dihydroxyvitamin D [4]. Epidemiological studies have established that vitamin D-deficiency is common in patients with IBD that is associated with increased risk of IBD [5,6,7,8,9,10,11,12,13,14], whereas high vitamin D intake lowers the risk of IBD [15].

We recently reported that colonic epithelial VDR levels are markedly reduced in patients with IBD [16]. VDR down-regulation is mainly caused by mucosal inflammation that is mediated by mircoRNA-346 [17]. Global genetic deletion of VDR in mice resulted in severe colitis and high mortality in experimental colitis models [18]. We also showed that transgenic mice that carry a villin promoter-driven FLAG-tagged human (h) VDR transgene were highly resistant to experimental colitis, and this epithelial hVDR transgene was able to rescue the global VDR-null mice from developing severe colitis and death [16]. Together our studies demonstrated that the VDR signaling in IECs prevents mucosal inflammation and inhibits colitis by protecting the integrity of the mucosal barrier, and this anti-colitic activity of the epithelial VDR appears to be independent of the VDR activity from the non-epithelial immune cells [16].

Interleukin (IL)-10-null mice spontaneously develop chronic intestinal inflammation due to aberrant immune response [19,20], and this model is widely used in colitis research. Previous studies showed that vitamin D-deficiency exacerbated colitis, whereas dietary vitamin D supplementation ameliorated colitis in this IL-10 knockout (KO) model [21]. IL-10KO mice that also carry VDR deletion (i.e. IL-10/VDR double knockout mice) developed more severe colitis compared to IL-10KO mice [22]; however, because VDR deletion is global, it is unclear how the VDR signaling affected colitis development in this double knockout model. We reasoned that, if gut epithelial VDR prevents mucosal inflammation as a primary defense mechanism that is independent of the non-epithelial immune VDR actions, then VDR over-expression in the IECs should be able to inhibit mucosal inflammation caused by an aberrant immune system. In this study we used the IL-10KO model to test this hypothesis.

Materials and Methods

Animals

Villin promoter-driven FLAG-tagged hVDR transgenic (Tg) mice have been described previously [16]. IL-10KO mice were obtained from Jackson Laboratory (Stock # 002251). IL-10KO mice that expressed the FLAG-hVDR transgene (designated as IL-10KO/Tg) were obtained from crossing IL-10KO and villin-hVDR-Tg mice. IL-10KO and IL-10KO/Tg mice were studied in parallel and followed for up to 25 weeks. Development of prolapse was recorded. The intestine was harvested at 1, 3 and 6 months of age. Colonic mucosal lysates and total RNAs were prepared. Colons were subjected to histological and immunohistological analyses. All animal studies were approved by the Institutional Animal Care and Use Committee at The University of Chicago.

Histology and immunostaining

Freshly harvested colons were fixed in 10% formalin overnight and processed as described previously [16]. Colonic histology was examined by hematoxylin and eosin staining. Histological scores were recorded based on a previously described scoring system as described [16]. To assess T cell infiltration, colon sections were stained with anti-CD4 antibodies (BD Biosciences), followed by FITC-conjugated secondary anti-IgG antibodies. Staining was visualized under a fluorescent microscope (Olympus).

RT-PCR

Mucosal total RNAs were extracted using TRIzol reagents (Life Technologies). First-strand cDNAs were synthesized using a ThermoScript RT kit (Life Technologies). Mucosal cytokine transcripts were quantified by real time RT-PCR in a Roche 480 Real-Time PCR System, using SensiFAST SYBR No-Rox kits (Bioline). The amount of transcripts was calculated using the 2^{-C_t} formula, normalized to the endogenous GAPDH levels. PCR primers were as described previously [16,17].

Western blot

Mucosal lysates were dissolved in Laemmli sample buffer for Western blotting analysis. Proteins were separated by SDS-PAGE and electroblotted onto Immobilon-P membranes. The membranes were incubated with primary antibodies, followed by horseradish peroxidase-conjugated secondary antibodies. Antigens were visualized using a chemiluminescence kit (Thermo Scientific) as described [16]. The primary antibodies against the following proteins were used: VDR, FLAG (Santa Cruz Biotechnology), PUMA (Abcam), caspase 3 and p53 (Cell Signaling) and -actin (Sigma-Aldrich). The relative amount of proteins was quantified using the gel analysis software UNSCAN-IT Gel version 5.3 (Silk Scientific, Orem, UT).

Myeloperoxidase (MPO) activity

Mucosal lysate MPO activity was measured according to the method previously published [23].

Statistical analysis

Data values were presented as means SD. Statistical comparisons were carried out using unpaired two-tailed Student's *t*-test or one-way ANOVA as appropriate, with $P < 0.05$ being considered statistically significant.

Results

We identified IL-10KO/Tg mice by genomic DNA genotyping, and confirmed their genotype by Western blot analysis of colonic mucosal lysates, using antibodies against FLAG and VDR (Fig. 1A). As expected, both FLAG-tagged hVDR and endogenous VDR were detected in IL-10KO/Tg mice, but the FLAG-hVDR transgene was not detectable in either wild-type or IL-10KO mice. Interestingly, the endogenous intestinal mucosal VDR level in the IL-10KO mice (at three months of age) was markedly reduced compared to wild-type mice. In fact, we found that mucosal VDR levels were also down-regulated in IL-10KO mice at two months of age, but the reduction was less dramatic than that seen in 3 month old IL-10KO mice (data not shown). Mucosal inflammation increases in IL-10KO mice with age. So these observations are consistent with our previous speculation that inflammation suppresses mucosal epithelial VDR expression [16]. In fact, we confirmed recently that intestinal epithelial VDR is down-regulated by TNF- α by a microRNA mediated mechanism [17]. IL-10KO mice developed prolapse with aging (Fig. 1B). Within 25 weeks accumulatively 79% of IL-10KO mice developed prolapse, whereas only 40% IL-10KO/Tg mice did so ($P < 0.001$) (Fig. 1C). Morphologically the large intestine of IL-10KO/Tg mice appeared longer than that of IL-10KO mice at 3 months of age (Fig. 2A). Histological examinations showed marked crypt hyperplasia (Fig. 2B) and massive immune cell infiltration in the lamina propria in the colon of IL-10KO mice (Fig. 2B), and immunostaining confirmed dramatic infiltration of CD4⁺ T lymphocytes in both the proximal and distal colons of IL-10KO mice (Fig. 2C). These colonic abnormalities, however, were markedly ameliorated in IL-10KO/Tg mice (Fig. 2B and 2C).

We further quantified the colonic damage and colonic inflammation. The histological score of the IL-10KO/Tg colon was substantially and significantly reduced compared to IL-10KO mice at 6 months of age (Fig. 3A). Mucosal myeloperoxidase (MPO) activity, which measures neutrophil presence in the lamina propria, was significantly lower in IL-10KO/Tg mice relative to IL-10KO littermates at 1, 3 and 6 months of age (Fig. 3B). Moreover, the transcript levels of mucosal pro-inflammatory cytokines and chemokines, including TNF- α , IL-6, IL-1, IL-17, IL-18, INF- γ , and MIP2, were also significantly attenuated in IL-10KO/Tg mice at 6 months compared to IL-10KO counterparts (Fig. 3C). These data demonstrated that IL-10KO/Tg mice were much less inflamed in the colon.

Our previous studies showed that gut epithelial VDR signaling protects the mucosal barrier integrity by inhibiting IEC apoptosis in the 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced colitis model, as the epithelial VDR action targets PUMA [16], a key pro-apoptotic regulator of IEC apoptosis [24]. Here we found that, similar to our early observation in the TNBS model, mucosal PUMA expression was significantly suppressed in IL-10KO/Tg mice compared to IL-10KO mice (Fig. 4A and B), whereas mucosal p53 expression was not significantly different between these two genotypes (Fig. 4C and E). Consistently, mucosal

caspase 3 activation was markedly suppressed in IL-10KO/Tg mice compared to IL-10KO mice (Fig. 4C and D). These observations suggest that transgenic VDR expression in IECs of IL-10KO mice inhibits PUMA-mediated IEC apoptosis, thus protecting the mucosal barrier. This results in suppression of mucosal inflammation caused by IL-10 deficiency.

Discussion

The intestine, especially the colon, contains an enormous amount of commensal bacteria that can cause colonic inflammation if the intestinal homeostasis is disrupted. IL-10 is a key anti-inflammatory cytokine that plays an essential role in the maintenance of this hemostasis. It has been shown that IL-10 targets both the innate and adaptive immune cells in the colon, including macrophages, Treg cells and IL-17⁺CD4⁺ T cells, to suppress immune response [25,26,27]. Therefore, IL-10-deficiency causes Th1 cell-mediated intestinal inflammation that leads to the development of colitis in mice and humans [19,20,28,29]. Notably, the presence of commensal bacteria is required for the development of colitis in IL-10-null mice [30], suggesting the importance of the mucosal barrier in the control of mucosal inflammation caused by aberrant immune responses.

In this study we studied the effects of epithelial VDR actions on mucosal inflammation caused by IL-10-deficiency. Our rationale is that, if VDR overexpression in the IECs is able to strengthen the mucosal barrier as we have reported recently [16], then the hVDR transgene should be able to attenuate the severity of colitis caused by an impaired immune system. Indeed we showed that when the hVDR transgene is targeted to the IECs in IL-10KO mice, the prolapse incident and mucosal inflammation were markedly reduced compared to the parental IL-10KO mice. Consequently, crypt hyperplasia, immune cell infiltration and production of pro-inflammatory cytokines and chemokines in the colon were also attenuated in IL-10KO/Tg mice. Although here we have only quantified the mRNA transcripts of these cytokines, it is highly likely that the transcript levels reflect the protein levels of these cytokines. We also observed that apoptotic pathways induced by inflammation, including the induction of PUMA, a key pro-apoptotic regulator in colonic epithelial cells [24], and caspase 3 activation, were blocked in the colon of IL-10KO/Tg mice, consistent with our previous conclusion that epithelial VDR signaling inhibits colonocyte apoptosis by targeting the NF- κ B-PUMA pathway [16]. Indeed, the epithelial originality of the PUMA pro-apoptotic pathway in the promotion of intestinal epithelial cell apoptosis has been well established [24]. Together these data confirm that the epithelial VDR plays a key role in protecting the mucosal barrier from inflammation-inflicted injury. When the mucosal barrier is strengthened by VDR overexpression in the IECs [16], it can more effectively prevent the invasion of luminal commensal bacteria and antigens, thus reducing mucosal inflammation regardless of the status of the immune system. In other words, as the robust mucosal inflammation occurring in IL-10 null mice requires commensal bacterial invasion, epithelial VDR signaling attenuates mucosal inflammation by strengthening the mucosal barrier, thus reducing the chance of bacterial interaction with the IL-10-null hyperactive immune system.

In our previous studies we already used a number of experimental models to demonstrate an inhibitory role of epithelial VDR signaling in the pathogenesis of colitis. These are colitis

models induced by dextran sulfate sodium (SDS), TNBS or adoptive T cell transfer [16]. In the DSS model, DSS damages the mucosal epithelium, and colitis occurs as a result of the disruption of the mucosal epithelial barrier. This model is thought to resemble ulcerative colitis in humans [31]. In the TNBS model, TNBS can haptenize colonic autologous or microbial proteins, rendering them immunogenic to the host immune system and thus triggering T-cell mediated colonic inflammation [32]. The TNBS model is believed to resemble Crohn's disease in humans. The adoptive T cell transfer model is a chronic colitis model created by a transfer of CD4⁺CD45RB^{high} T cells into a Rag(−/−) background. In this model colitis is induced as a result of a disruption of T cell homeostasis [33]. Here in the present study we used IL-10-deficient model, which is very different from the three models we used previously. This model develops widespread colitis spontaneously with age because of an ill regulated immune system caused by IL-10 deficiency. Although defects in mucosal barrier permeability are detected in IL-10-deficient mice before the histological injury develops, this barrier problem is not originated from epithelial cells, but from a dysregulated immune system [34]. Therefore, in IL-10-deficient mice colitis is not directly caused by a defect in the mucosal epithelial barrier, where the hVDR transgene is expressed, which further underscores the importance of the epithelial VDR signaling in the inhibition of colitis. This work, together with our previous studies outlined above [16], provides compelling evidence for a critical mucosal barrier-protecting role of intestinal epithelial VDR. The presence of VDR in gut epithelial cells is essential for maintaining the integrity of the mucosal barrier.

Together our studies reveal a mechanism whereby mucosal inflammation is reduced by epithelial VDR signaling. They suggest that VDR could be a target in the therapy of inflammatory bowel diseases. By raising epithelial VDR levels, either through vitamin D therapy or anti-TNF therapy, the mucosal epithelial barrier can be strengthened. However, given the wide expression of VDR in virtually all cells within the colon, VDR actions to inhibit mucosal inflammation may not limit to barrier protection. For example, VDR signaling may control the profiles and diversity of the microbiota or influence the interaction between commensal bacteria and intestinal epithelial cells by regulating anti-microbial peptide production [35,36]. VDR signaling may also directly regulate the innate and adaptive immune systems, as vitamin D hormone as an immune modulatory agent has been well established [37,38]. Therefore, how VDR signaling influences the microbiota and control the immune system in the context of mucosal inflammation warrants further investigation.

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Abbreviations

VDR	vitamin D receptor
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IBD	inflammatory bowel diseases
CD	Crohn's disease
UC	Ulcerative colitis
Interleukin 10	IL-10
PUMA	p53-upregulated modulator of apoptosis
TNBS	2,4,6-trinitrobenzenesulfonic acid
DSS	dextran sulfate sodium

References

1. Salim SY, Soderholm JD. Importance of disrupted intestinal barrier in inflammatory bowel diseases. *Inflamm Bowel Dis*. 2011; 17:362–381. [PubMed: 20725949]
2. Fasano A, Shea-Donohue T. Mechanisms of disease: the role of intestinal barrier function in the pathogenesis of gastrointestinal autoimmune diseases. *Nat Clin Pract Gastroenterol Hepatol*. 2005; 2:416–422. [PubMed: 16265432]
3. Gibson PR. Increased gut permeability in Crohn's disease: is TNF the link? *Gut*. 2004; 53:1724–1725. [PubMed: 15542502]
4. Haussler MR, Whitfield GK, Kaneko I, Haussler CA, Hsieh D, et al. Molecular mechanisms of vitamin D action. *Calcif Tissue Int*. 2013; 92:77–98. [PubMed: 22782502]
5. Loftus EV Jr. Clinical epidemiology of inflammatory bowel disease: Incidence, prevalence, and environmental influences. *Gastroenterology*. 2004; 126:1504–1517. [PubMed: 15168363]
6. Lim WC, Hanauer SB, Li YC. Mechanisms of Disease: vitamin D and inflammatory bowel disease. *Nature Clinical Practice Gastroenterology & Hepatology*. 2005; 2:308–315.
7. Bischoff SC, Herrmann A, Goke M, Manns MP, von zur Muhlen A, et al. Altered bone metabolism in inflammatory bowel disease. *Am J Gastroenterol*. 1997; 92:1157–1163. [PubMed: 9219790]
8. Jahnsen J, Falch JA, Mowinkel P, Aadland E. Vitamin D status, parathyroid hormone and bone mineral density in patients with inflammatory bowel disease. *Scand J Gastroenterol*. 2002; 37:192–199. [PubMed: 11843057]
9. Marx G, Seidman EG. Inflammatory bowel disease in pediatric patients. *Curr Opin Gastroenterol*. 1999; 15:322–325. [PubMed: 17023965]
10. Pappa HM, Gordon CM, Saslowsky TM, Zholudev A, Horr B, et al. Vitamin D status in children and young adults with inflammatory bowel disease. *Pediatrics*. 2006; 118:1950–1961. [PubMed: 17079566]
11. Scharla SH, Minne HW, Lempert UG, Leidig G, Hauber M, et al. Bone mineral density and calcium regulating hormones in patients with inflammatory bowel disease (Crohn's disease and ulcerative colitis). *Exp Clin Endocrinol*. 1994; 102:44–49. [PubMed: 8005208]
12. Schulte CM. Review article: bone disease in inflammatory bowel disease. *Aliment Pharmacol Ther*. 2004; 20(Suppl 4):43–49. [PubMed: 15352893]
13. Sinnott BP, Licata AA. Assessment of bone and mineral metabolism in inflammatory bowel disease: case series and review. *Endocr Pract*. 2006; 12:622–629. [PubMed: 17229657]
14. Souza HN, Lora FL, Kulak CA, Manas NC, Amarante HM, et al. Low levels of 25-hydroxyvitamin D (25OHD) in patients with inflammatory bowel disease and its correlation with bone mineral density. *Arq Bras Endocrinol Metabol*. 2008; 52:684–691. [PubMed: 18604382]
15. Ananthakrishnan AN, Khalili H, Higuchi LM, Bao Y, Korzenik JR, et al. Higher predicted vitamin D status is associated with reduced risk of Crohn's disease. *Gastroenterology*. 2012; 142:482–489. [PubMed: 22155183]
16. Liu W, Chen Y, Golan MA, Annunziata ML, Du J, et al. Intestinal epithelial vitamin D receptor signaling inhibits experimental colitis. *J Clin Invest*. 2013; 123:3983–3996. [PubMed: 23945234]

17. Chen Y, Du J, Zhang Z, Liu T, Shi Y, et al. MicroRNA-346 Mediates Tumor Necrosis Factor- α -induced Down-Regulation of Gut Epithelial Vitamin D Receptor in Inflammatory Bowel Diseases. *Inflammatory Bowel Diseases*. 2014 in press.
18. Kong J, Zhang Z, Musch MW, Ning G, Sun J, et al. Novel role of the vitamin D receptor in maintaining the integrity of the intestinal mucosal barrier. *Am J Physiol Gastrointest Liver Physiol*. 2008; 294:G208–216. [PubMed: 17962355]
19. Kuhn R, Lohler J, Rennick D, Rajewsky K, Muller W. Interleukin-10-deficient mice develop chronic enterocolitis. *Cell*. 1993; 75:263–274. [PubMed: 8402911]
20. Berg DJ, Davidson N, Kuhn R, Muller W, Menon S, et al. Enterocolitis and colon cancer in interleukin-10-deficient mice are associated with aberrant cytokine production and CD4(+) TH1-like responses. *J Clin Invest*. 1996; 98:1010–1020. [PubMed: 8770874]
21. Cantorna MT, Munsick C, Bemiss C, Mahon BD. 1,25-Dihydroxycholecalciferol prevents and ameliorates symptoms of experimental murine inflammatory bowel disease. *J Nutr*. 2000; 130:2648–2652. [PubMed: 11053501]
22. Froicu M, Weaver V, Wynn TA, McDowell MA, Welsh JE, et al. A crucial role for the vitamin D receptor in experimental inflammatory bowel diseases. *Mol Endocrinol*. 2003; 17:2386–2392. [PubMed: 14500760]
23. Kong J, Zhu X, Shi Y, Liu T, Chen Y, et al. VDR attenuates acute lung injury by blocking Ang-2-Tie-2 pathway and renin-angiotensin system. *Mol Endocrinol*. 2013; 27:2116–2125. [PubMed: 24196349]
24. Qiu W, Wu B, Wang X, Buchanan ME, Regueiro MD, et al. PUMA-mediated intestinal epithelial apoptosis contributes to ulcerative colitis in humans and mice. *J Clin Invest*. 2011; 121:1722–1732. [PubMed: 21490394]
25. Takeda K, Clausen BE, Kaisho T, Tsujimura T, Terada N, et al. Enhanced Th1 activity and development of chronic enterocolitis in mice devoid of Stat3 in macrophages and neutrophils. *Immunity*. 1999; 10:39–49. [PubMed: 10023769]
26. Chaudhry A, Samstein RM, Treuting P, Liang Y, Pils MC, et al. Interleukin-10 signaling in regulatory T cells is required for suppression of Th17 cell-mediated inflammation. *Immunity*. 2011; 34:566–578. [PubMed: 21511185]
27. Huber S, Gagliani N, Esplugues E, O'Connor W Jr, Huber FJ, et al. Th17 cells express interleukin-10 receptor and are controlled by Foxp3(–) and Foxp3+ regulatory CD4+ T cells in an interleukin-10-dependent manner. *Immunity*. 2011; 34:554–565. [PubMed: 21511184]
28. Glocker EO, Frede N, Perro M, Sebire N, Elawad M, et al. Infant colitis--it's in the genes. *Lancet*. 2010; 376:1272. [PubMed: 20934598]
29. Kotlarz D, Beier R, Murugan D, Diestelhorst J, Jensen O, et al. Loss of interleukin-10 signaling and infantile inflammatory bowel disease: implications for diagnosis and therapy. *Gastroenterology*. 2012; 143:347–355. [PubMed: 22549091]
30. Sellon RK, Tonkonogy S, Schultz M, Dieleman LA, Grenther W, et al. Resident enteric bacteria are necessary for development of spontaneous colitis and immune system activation in interleukin-10-deficient mice. *Infect Immun*. 1998; 66:5224–5231. [PubMed: 9784526]
31. Okayasu I, Hatakeyama S, Yamada M, Ohkusa T, Inagaki Y, et al. A novel method in the induction of reliable experimental acute and chronic ulcerative colitis in mice. *Gastroenterology*. 1990; 98:694–702. [PubMed: 1688816]
32. Neurath MF, Fuss I, Kelsall BL, Stuber E, Strober W. Antibodies to interleukin 12 abrogate established experimental colitis in mice. *J Exp Med*. 1995; 182:1281–1290. [PubMed: 7595199]
33. Ostanin DV, Bao J, Koboziev I, Gray L, Robinson-Jackson SA, et al. T cell transfer model of chronic colitis: concepts, considerations, and tricks of the trade. *Am J Physiol Gastrointest Liver Physiol*. 2009; 296:G135–146. [PubMed: 19033538]
34. Madsen KL, Malfair D, Gray D, Doyle JS, Jewell LD, et al. Interleukin-10 gene-deficient mice develop a primary intestinal permeability defect in response to enteric microflora. *Inflamm Bowel Dis*. 1999; 5:262–270. [PubMed: 10579119]
35. Lagishetty V, Misharin AV, Liu NQ, Lisse TS, Chun RF, et al. Vitamin D deficiency in mice impairs colonic antibacterial activity and predisposes to colitis. *Endocrinology*. 2010; 151:2423–2432. [PubMed: 20392825]

36. Hewison M. Antibacterial effects of vitamin D. *Nat Rev Endocrinol*. 2011; 7:337–345. [PubMed: 21263449]
37. Mora JR, Iwata M, von Andrian UH. Vitamin effects on the immune system: vitamins A and D take centre stage. *Nat Rev Immunol*. 2008; 8:685–698. [PubMed: 19172691]
38. Hart PH, Gorman S, Finlay-Jones JJ. Modulation of the immune system by UV radiation: more than just the effects of vitamin D? *Nat Rev Immunol*. 2011; 11:584–596. [PubMed: 21852793]

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Figure 1.

VDR overexpression in intestinal epithelial cells reduces prolapse development. (A) Western blot analysis of mucosal lysates from wild-type (WT), IL-10KO and IL-10KO/Tg mice at 3 months of age using anti-VDR and anti-FLAG antibodies. Note the marked reduction of mucosal VDR in IL-10KO mice. (B) Photo images of a normal mouse and a mouse with prolapse (indicated by an *arrow*). (C) Accumulative percentage of IL-10KO and IL-10KO/Tg mice that developed prolapse over time in 25 weeks after birth. $n > 20$ in each group. $P < 0.001$ by log-rank test.

Figure 2. Epithelial VDR overexpression reduces colonic crypt hyperplasia and lamina propria lymphocyte infiltration. (A) Morphology of the large intestines from IL-10KO and IL-10KO/Tg mice at 3 months of age. (B) Representative H&E stained colon sections from IL-10KO and IL-10KO/Tg mice at 3 months of age. *Arrows* indicate examples of infiltrated immune cells at 400 $\times$. (C) Anti-CD4 immunostaining of proximal and distal colon sections from IL-10KO and IL-10KO/Tg mice. Note the marked CD4⁺ cell infiltration in the lamina propria in IL-10KO colon.

Figure 3.

The hVDR transgene reduces colonic inflammation. (A) Semi-quantitative histological scores of IL-10KO and IL-10KO/Tg mice. *** $P < 0.001$ vs. IL-10KO, $n = 5-6$. (B) Colonic mucosal myeloperoxidase (MPO) activity in IL-10KO and IL-10KO/Tg mice at 1, 3 and 6 months of age. * $P < 0.05$, ** $P < 0.01$ vs. IL-10KO at the same age, $n = 5-7$. (C) Real time RT-PCR quantitation of pro-inflammatory cytokines and chemokines in colonic mucosa from IL-10KO and IL-10KO/Tg mice at 6 months of age. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ between IL-10KO and IL-10KO/Tg; $n = 6$.

Figure 4. Epithelial VDR overexpression attenuates apoptotic signaling. (A and B) Western blot analysis (A) and its densitometric quantitation (B) of mucosal PUMA protein levels in IL-10KO and IL-10KO/Tg mice. * $P < 0.05$ vs. IL-10KO; (C-E) Western blot analyses (C) and densitometric quantitation of colonic mucosal caspase 3 cleavage (D) and p53 protein (E) in IL-10KO and IL-10KO/Tg mice. *** $P < 0.001$ vs. IL-10KO.