Ornithine Decarboxylase in Macrophages Exacerbates Colitis and Promotes Colitis-Associated Colon Carcinogenesis by Impairing M1 Immune Responses

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Abstract

Ornithine decarboxylase (ODC) is the rate-limiting enzyme for polyamine biosynthesis and restricts M1 macrophage activation in gastrointestinal (GI) infections. However, the role of macrophage ODC in colonic epithelial-driven inflammation is unknown. Here, we investigate cell-specific effects of ODC in colitis and colitis-associated carcinogenesis (CAC). Human colonic macrophages expressed increased ODC levels in active ulcerative colitis and Crohn’s disease, colitis-associated dysplasia, and CAC. Mice lacking Odc in myeloid cells (Odcmye mice) that were treated with dextran sulfate sodium (DSS) exhibited improved survival, body weight, and colon length and reduced histologic injury versus control mice. In contrast, GI epithelial-specific Odc knockout had no effect on clinical parameters. Despite reduced histologic damage, colitis tissues of Odcmye mice had increased levels of multiple proinflammatory cytokines and chemokines and enhanced expression of M1, but not M2 macrophages. Increased levels of histone 3, lysine 9 acetylation, a marker of open chromatin, were manifest in tumor macrophages of Odcmye mice, consistent with our findings that macrophage ODC affects histone modifications that upregulate M1 gene transcription during GI infections. These findings support the concept that macrophage ODC augments epithelial injury-associated colitis and CAC by impairing the M1 responses that stimulate epithelial repair, antimicrobial defense, and antitumoral immunity. They also suggest that macrophage ODC is an important target for colon cancer chemoprevention.

Significance: Ornithine decarboxylase contributes to the pathogenesis of colitis and associated carcinogenesis by impairing M1 macrophage responses needed for antitumoral immunity; targeting ODC in macrophages may represent a new strategy for chemoprevention. Cancer Res; 78(15); 4303–15.

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Introduction

M1 macrophage activation is essential in the host defense response to microbes and to tumor cells (1, 2). M2 macrophages have a key role in parasite defense, and are associated with wound healing (3). There has been a long interest in the role of M1 versus M2 macrophages in the tumor microenvironment (2, 4), but as yet this has not led to therapeutic interventions.

In inflammatory bowel disease (IBD), there is a strong risk for colitis-associated carcinogenesis (CAC) that results from the chronic inflammatory state (5, 6). Indeed, CAC occurs in 20% of patients with IBD, and mortality rates can exceed 50% (7, 8). However, despite this clinical problem, the mainstay of clinical management is annual surveillance colonoscopy. Accordingly, insight into the pathogenesis of CAC, and new therapeutic strategies, are urgently needed. In particular, the role of the host immune response in CAC, and especially the roles of M1 and M2 macrophages are not understood.

We have investigated the role of macrophage activation patterns, often referred to M1 versus M2 polarization, in host–pathogen interactions in the gastrointestinal (GI) mucosa (1, 9, 10). One key regulatory component of macrophage function revealed by these studies has been the role of polyamines (10, 11). Polyamine biosynthesis begins with the production of the biogenic amine, putrescine, which is produced by the enzyme ornithine decarboxylase (ODC1, hereafter ODC; refs. 12, 13).
Putrescine is then converted to the polyamines spermidine and spermine by the action of constitutively active synthases (13). We recently reported that the myeloid-specific deletion of Odc (Odc<sup>emy</sup>) provokes a marked increase in the response of mouse bone marrow-derived macrophages to M1 stimuli including Helicobacter pylori, Citrobacter rodentium, or LPS + IFNγ, and increased expression of M1 genes (10). In addition, in mouse models of gastric inflammation induced by H. pylori infection, and of colon inflammation induced by C. rodentium infection, Odc<sup>emy</sup> mice have increased M1 responses and production of M1 cytokines and chemokines. However, when such bone marrow-derived Odc<sup>emy</sup> macrophages are exposed to M2 stimulation with IL4, there is also an upregulation of M2 gene expression (10). Thus, the ODC-polyamine circuit can harness both M1 and M2 macrophage responses. Finally, these effects were linked to histone modifications (10), where Odc<sup>emy</sup> macrophages have increases in histone marks that are hallmarks of open chromatin (e.g., H3K4 monomethylation and H3K9 acetylation), leading to increased transcription (10, 14).

Macrophage-driven innate immune responses are major contributors to the pathogenesis of human ulcerative colitis (UC; refs. 15–17) and to related mouse models of colitis and CAC (1, 16, 18). Given this, we assessed the role of ODC in UC and CAC. Herein, we report that ODC expression is increased in human macrophages of IBD tissues displaying active colitis, dysplasia, and CAC. Strikingly, in both dextran sulfate sodium (DSS)-induced colitis and the azoxymethane (AOM)-DSS model of CAC, myeloid-selective loss of Odc reduces clinical disease and tumorigenesis, and this is linked to increases in the M1 response. Thus, in both an epithelial injury model and in associated carcinogenesis, M1 macrophage functions are beneficial, and ODC acts to impair this response, providing new insights into strategies for colon cancer prevention.

**Materials and Methods**

**Animal studies**

Animal experiments were approved by the Vanderbilt University Institutional Animal Care and Use Committee and the Research and Development Committee of the Veterans Affairs Tennessee Valley Healthcare System. Odc<sub>fl/fl</sub> mice, and Odc<sub>emy</sub> mice generated by crossing Odc<sub>fl/fl</sub> with Lyz2<sup>cre/cre</sup> mice, were previously described (10). Odc<sub>emy</sub> mice were generated by crossing Odc<sub>fl/fl</sub> with Foxa3<sup>cre/cre</sup> mice (19, 20). Male mice between the ages of 7 and 12 weeks were used for all studies.

For the acute DSS colitis model, animals were treated with 2.5% DSS in the drinking water for 5 days followed by a 5-day recovery period. Control mice received normal drinking water for 10 days. For the colitis-associated cancer (CAC) model, mice first received one intraperitoneal injection of AOM (12.5 mg/kg) and were then treated three times with 4% DSS for 5 days beginning at days 5, 26, and 47, all as described (20).

Animals were monitored daily and mice that showed extreme distress, became moribund, or lost more than 20% of initial body weight were euthanized. After sacrifice, colons were removed, measured, cut longitudinally, cleaned, weighed, and Swiss-rolled for histology. Two proximal and distal 2 mm pieces were used for RNA and protein extraction. All histologic assessments were made in a blinded manner by a pathologist (M.K.W.). In the AOM-DSS model, tumors were counted and measured under a dissecting microscope; tumor and nontumor tissues were collected for RNA and protein extraction. Colons were then Swiss-rolled for histology.

**Isolation of myeloid cells and RNA analyses**

RNA from colonic tissues was extracted using the RNeasy Mini Kit (Qiagen; ref. 21). CD11b<sup>+</sup>, CD11c<sup>+</sup>, F4/80<sup>+</sup>, and Gr1<sup>+</sup> cells were positively selected (21) using biotinylated antibodies, streptavidin conjugated with magnetic beads, and IMagnet columns (BD Biosciences). RNA from cells was isolated using the 5 PRIME PerfectPure RNA 96 Cell CS Kit (21). Total RNA (1 μg) was reverse transcribed using an iScript CDNA Synthesis Kit (Bio-Rad) and oligo(dT) primers. Each real-time reverse transcription (RT)-PCR was performed with 2 μl of cDNA, iQ SYBR Green Supermix (Bio-Rad), and primers in Supplementary Table S1.

**Immunofluorescence and immunohistochemistry**

Sections (5 μm) from formalin-fixed paraffin-embedded Swiss-rolled murine tissues were deparaffinized and treated with citrate buffer for antigen retrieval. An IBD-associated cancer tissue microarray (TMA) from Vanderbilt University Medical Center, which contained cases of normal tissue, inactive and active UC and Crohn’s disease, dysplasia, and CAC, was used to assess ODC expression. Immunofluorescence for CD68, ODC, nitric oxide synthase 2 (NOS2), arginase 1 (ARG1), and H3K9Ac, and IHC for IFNγ, and IFNγ in tissues were measured using R&D DuoSet ELISA Kits (10).

**Measurement of cytokines and chemokines**

Colonic tissues were lysed in Cell Lytic Mammalian Tissue Lysis Extraction Reagent (Sigma) and analyzed using the 25-analyte MILLIPLEX MAP Mouse Cytokine/Chemokine Magnetic Bead Panel (EMD Millipore) on a FLEXMAP 3D instrument (Luminex; refs. 21, 22). Data were standardized to tissue protein concentrations measured by the BCA Protein Assay Kit (Pierce). TNFα and IFNγ in tissues were measured using R&D DuoSet ELISA Kits (10).

**Flow cytometry**

Immune cells from nontumor and tumor areas were isolated using Liberase DL and TL (23). Cells were stained for CD11b (Supplementary Table S2), washed, fixed, and permeabilized with CytoFix/CytoPerm (BD Biosciences). NOS2 or ARG1 antibodies were used (Supplementary Table S2). Data acquisition was performed using FlowJo software (Tree Star).

**Macrophage differentiation and measurement of polyamines**

Bone-marrow-derived macrophages (BMMacs) were differentiated, placed in DMEM supplemented with 2% BSA, and activated with IFNγ (200 units/mL) and LPS (10 ng/mL) for 24 hours (10). Polyamines were quantified by LC/MS in cell pellets (10).

**Statistical analysis**

Data are shown as the mean ± SEM. Statistical analyses were performed with Prism version 5.0c (GraphPad Software). Student t-test was performed for comparison between two groups. ANOVA with the Student–Newman–Keuls post hoc multiple comparisons test was performed to compare multiple groups. When data were not normally distributed, log transformation was performed. Survival was analyzed by the log-rank (Mantel–Cox) test.
Results

Patients with active UC and CAC have increased ODC expression in macrophages

To assess macrophage infiltration and ODC expression in patients with UC, as well as precancerous and cancerous colon lesions, a TMA from Vanderbilt University Medical Center was utilized. Histopathological diagnoses were established from clinical case material from surgical resections, and confirmed by hematoxylin and eosin staining of the cores on the TMA. Immunofluorescence staining was performed for the macrophage marker CD68, as well as ODC. Notably, this demonstrated that high levels of ODC are expressed in macrophages found in active colitis, dysplasia, and carcinoma (Fig. 1A). When scored by whole slide scanning and CellProfiler image analysis, there were marked increases in the percentage of CD68+/ODC+ cells per core (Fig. 1B), as well as an increase in the percentage of CD68+/ODC+ cells per CD68+ cells (Fig. 1C) in the colon of patients with active colitis, dysplasia, and CAC compared with tissues from normal individuals or to tissues from inactive UC. ODC staining was also detected in epithelial cells; however, there was no significant increase in ODC activity in macrophages and epithelial cells to UC and CAC, we used validated fluorescent staining was performed for the macrophage marker macrophage ODC contributes to DSS-induced colonic inflammation

To investigate the relative contribution of ODC in macrophages and epithelial cells to UC and CAC, we used validated mouse experimental colitis and CAC models. Odc expression was elevated in colonic tissues of mice with DSS-induced colitis, and AOM-DSS–induced CAC lesions (Supplementary Fig. S2A and S2B). There was a significant increase of Odc mRNA levels in cells positive for the markers CD11b (myeloid cells) and F4/80 (macrophages), but not CD11c (dendritic cells) or Gr1 (neutrophils and myeloid-derived suppressor cells) from the colon of DSS-treated mice (Supplementary Fig. S2C). To further assess the role of macrophage ODC in an epithelial injury and repair model of colitis induced by DSS, we used Odc−/− (Odcfl/fl)/Lyz2cre/cre mice, in which we demonstrated reduction in ODC protein expression in macrophages (10). At baseline, colony of Odc−/− mice are histologically normal, and do not differ from the control Odcfl/fl mice (Supplementary Fig. S3A). There were no changes in ODC expression in the epithelium of Odc−/− versus Odcfl/fl mice (Supplementary Fig. S3B). Loss of ODC activity in Odc−/− mice was verified by measuring polyamines in BMMacs. Upon stimulation with IFNγ + LPS, BMMacs from Odc−/−, but not Odc+/− mice, generated putrescine, the direct product of ODC, spermidine and spermine were not modulated by the M1 stimulus (Supplementary Fig. S3C). In mice treated with DSS for 5 days followed by 5 days of water, Odc−/− mice exhibited a significant improvement in survival (Fig. 2A). Odc−/− mice treated with DSS began to die on day 7 and by day 10, only 52% remained alive, whereas 88% of Odc+/− mice were alive at day 10. Body weight loss of DSS-treated Odc−/− mice was also significantly improved compared with DSS-treated Odc+/− mice (Fig. 2B), and colon length was less reduced in Odc−/− versus Odc+/− mice (Fig. 2C). Finally, histologic colitis was significantly improved in Odc−/− mice compared with Odc+/− mice (Fig. 2D and E).

Immunofluorescence analyses established that ODC protein expression was markedly elevated in DSS-treated Odc+/− versus untreated Odc+/− mice (Fig. 2F). Further, ODC expression was predominantly in macrophages, as evidenced by colocalization to cells marked by CD68 staining (Fig. 2F, left). As expected, ODC staining of CD68+ cells was absent in the colon tissues of DSS-treated Odc−/− mice (Fig. 2F, right). There was modest ODC staining of CD68+ colonic epithelial cells (Fig. 2F, right), which was not affected by myeloid-specific deletion of Odc. These data verify the specific knockout of ODC in colonic macrophages during DSS colitis.

Because ODC is also expressed in the colonic epithelial cells of patients with UC and CAC, we assessed the contribution of epithelial-derived ODC to the development of DSS-induced colitis, using Odc−/− mice. Expression of Odc mRNA (Supplementary Fig. S4A) and ODC protein (Supplementary Fig. S4B) was abolished in colonic epithelial cells from Odc−/− versus Odc+/− mice. When subjected to DSS treatment, Odc−/− mice exhibited no differences in body weight loss, colon length, or histologic injury compared with Odc+/− mice (Supplementary Fig. S4C–S4F).

ODC regulates the mucosal proinflammatory response during acute colitis

To gain further insights into the mechanism by which macrophage ODC contributes to the development of colitis, Luminex multiplex arrays were used to assess effects of ODC on cytokines and chemokines produced in the colonic mucosa. Despite the reduced histologic injury scores, in Odc−/− versus Odc+/− colon tissues from DSS-treated mice there were significant increases (Fig. 3A) in the innate cytokines CSF2 (GM-CSF), TNFα, IL-1α, and IL-1β, as well as the prototype Th1 cytokine IFNγ, and the chemokines CCL2 (MP2), CXCL9 (MIG), CXCL10 (IP-10), CCL2 (MCP-1), CCL3 (MIP-1α), and CCL5 (RANTES). Moreover, there was increased mRNA expression of the M1 markers Nos2 and Il1b in colitis tissues from DSS-treated Odc−/− versus DSS-treated Odc+/− mice (Fig. 3B). To further investigate the source of cytokines, macrophages were isolated by positive selection and increased mRNA expression of Nos2, Tnfα, Il1b, Il12a, Ccl5, and Cxcl10 was demonstrated in DSS-treated Odc−/− versus Odc+/− mice (Supplementary Fig. S5). In contrast, expression levels of the M2 markers, Arg1 and Chi3l3, were comparable in colonic tissues of DSS-treated Odc−/− and Odc−/− mice (Fig. 3B).

Odc−/− mice are protected from CAC

Because Odc−/− mice are protected in the DSS colitis model and macrophage ODC expression was detected in patients with CAC, we reasoned that macrophage ODC may play a role in colon carcinogenesis. Odc−/− and Odc+/− mice were subjected to the 77-day AOM-DSS model of CAC. The survival rate of Odc−/− mice was significantly improved compared with the Odc+/− animals on the AOM-DSS protocol (Fig. 4A). With the first and second cycles of DSS, Odc−/− and Odc−/− mice showed similar weight loss and recovery with water (Fig. 4B), but with the third cycle of DSS, Odc−/− mice had less weight loss than Odc−/− mice, and better improvement during the recovery period (Fig. 4B). Consistent with this difference in body weights, Odc−/− mice had a significantly lower number of tumors per mouse (Fig. 4C) and lower
overall tumor burden (Fig. 4D) than Odc<sup>fl</sup> animals. However, there was no difference in histologic injury scores in the nontumor area between both type of mice (Fig. 4E), most likely due to the long-time gap between the last dose of DSS and sacrifice (28 days). Histologic assessment of the tumors revealed less high-grade dysplasia in Odc<sup>Dmye</sup> versus Odc<sup>fl/fl</sup> mice (Fig. 4F and G).

Figure 1.
Staining of human colon TMA. A, Representative hematoxylin and eosin staining and immunofluorescence for CD68 (red), ODC (green), and nuclei (DAPI, blue) in control and UC tissues from the TMA, depicted in yellow in these merged images. Boxes indicate areas of the images shown in higher magnification. Arrowheads, cells double-positive for CD68 and ODC. Scale bars, 50 µm. B and C, Quantification of CD68<sup>+</sup>ODC<sup>+</sup> cells among the total number of cells in each individual core (B), or among the total number of CD68<sup>+</sup> cells in each individual core (C), was determined using CellProfiler software. Normal subjects: 5 tissues were from the uninvolved normal area (open circles) and one tissue (red circle) was from a normal control case. For B and C, **, P < 0.01 and ****, P < 0.001 versus Normal; *, P < 0.05, **, P < 0.01, and $$$, P < 0.001 compared with inactive colitis.
Figure 2.
Effect of myeloid-specific Odc deletion on DSS-induced colitis. Mice were treated with 2.5% DSS for 5 days and given normal drinking water for the next 5 days. A, Survival was monitored daily. The Kaplan–Meier plot was generated from two separate experiments pooled together; n = 22 Odc<sup>fl/fl</sup> mice and n = 22 Odc<sup>mye</sup> mice. For this representation of the data, survival is defined as body weight loss of more than 20%. No death was observed in untreated animals. B, Body weights, presented as the percentage of initial body weight. *P < 0.05 and **P < 0.01 compared with DSS-treated Odc<sup>fl/fl</sup> animals. C, Colon length; *P < 0.05 compared with DSS-treated Odc<sup>fl/fl</sup> mice. D and E, Colon length were scored histologically from tissues stained with hematoxylin and eosin (E); *P < 0.05 compared with DSS-treated Odc<sup>fl/fl</sup> mice. Scale bars, 100 μm. F, Immunofluorescence for CD68 (red), ODC (green), and nuclei (blue) in colonic tissues; CD68<sup>+</sup> ODC<sup>+</sup> cells in the merged images are depicted in yellow. Scale bar, 100 μm.
Figure 3.
Quantification of cytokines and chemokines, as well as gene expression of M1 and M2 markers in murine colonic tissues. A, Concentrations of cytokines and chemokines in colon tissues were determined by Luminex assay. B, Analysis of Nos2, Il1b, Arg1, and Chil3 mRNA expression in colonic tissues. In A and B, *P < 0.05, **P < 0.01, and ***P < 0.001 versus Odcfl/fl mice.
Figure 4.
Effect of myeloid-specific Odc deletion on AOM-DSS-induced CAC. A, Survival was monitored daily; the Kaplan-Meier plot was performed from two separate experiments pooled together; n = 14 Odc<sup>fl/fl</sup> mice and n = 11 Odc<sup>Dmye</sup> mice. No death was observed in control animals. B, Percentage of initial body weight was determined at the indicated time points. C, Tumor number, determined using a dissecting microscope. D, Tumor burden, determined by the addition of the area of each tumor. E, Histologic colitis. F, Percentage of cases with low-grade dysplasia (LGD) and high-grade dysplasia (HGD). G, Representative hematoxylin and eosin-stained images from AOM-DSS-treated mice. Scale bars, 100 μm. For B-F, *, P < 0.05; **, P < 0.01, and ***, P < 0.001 compared with Odc<sup>fl/fl</sup> mice treated with AOM-DSS.
Loss of Odc enhances macrophage polarization towards M1 during experimental CAC

The treatment of mice with AOM-DSS led to increases in mRNA expression of the M1 markers Nos2, Il1b, Il12a, Il12b, and Tnfa, as well as the Th1 marker Ifng, in both the nontumor and tumor areas (Fig. 5A). Strikingly, all of these markers were significantly more expressed in the tumors of the OdcDmye versus Odcfl/fl mice (Fig. 5A). TNFα and IFNγ were also analyzed by ELISA from colonic tissues 77 days post-AOM injection. *P < 0.05, **P < 0.01, and ***P < 0.001 compared with untreated Odcfl/fl mice; †P < 0.05, ‡P < 0.01, and §§P < 0.001 compared with the tumors in Odcfl/fl mice.

To confirm these findings, we analyzed by flow cytometry the expression of the prototype M1 and M2 proteins, NOS2 and ARG1, respectively, in intratumoral macrophages. The numbers of NOS2+CD11b+ cells were significantly higher in tumors from OdcDmye mice versus tumors from Odcfl/fl animals (Fig. 6A), whereas no differences were observed in ARG1 expression in

Figure 5. M1 profile in nontumor and tumor tissues. A, mRNA levels of M1 markers Nos2, Il1b, Il12a, Il12b, Tnfa, and Ifng assessed by real-time RT-PCR. B, TNFα and IFNγ were also analyzed by ELISA from colonic tissues 77 days post-AOM injection. *P < 0.05, **P < 0.01, and ***P < 0.001 compared with untreated Odcfl/fl mice; †P < 0.05, ‡P < 0.01, and §§P < 0.001 compared with the tumors in Odcfl/fl mice.
CD11b⁺ cells (Fig. 6A). The composite data from multiple animals demonstrated significant increases in the percentage of NOS2⁺CD11b⁺ cells (Fig. 6B), but not ARG1⁺CD11b⁺ cells (Fig. 6C), in the tumors of AOM-DSS-treated Odc⁻/⁻ mice versus tumors from Odc⁺/⁻ mice. When quantified as number of cells per gram of colon tissue, a similar pattern of increased NOS2⁺ (Supplementary Fig. S7A), but not ARG1⁺ cells (Supplementary Fig. S7B) in the CD11b⁺ cells was demonstrated, resulting in an increased ratio of NOS2/ARG1 in the CD11b cells (Supplementary Fig. S7C). Similarly, by immunofluorescence, the level of ARG1 in CD68⁺ cells was not affected by Odc loss (Fig. 7A), whereas NOS2 was more abundant in CD68⁺ cells present in the tumors of Odc⁺/⁻ mice than in tumors of Odc⁻/⁻ animals (Fig. 7B). There were no differences in staining for the Treg marker, FOXP3, or the cytotoxic T cell marker, CD8, in tumors of Odc⁺/⁻ versus Odc⁻/⁻ mice (Supplementary Fig. S8).

In GI infection models, Odc loss in macrophages provokes increases in histone marks that are typical of open chromatin and that are associated with the increased expression of genes encoding M1 markers (NOS2 and cytokines), as described (10). Notably, macrophage levels of the euchromatin histone mark H3K9ac, which is associated with M1 gene expression (10), were augmented in the tumors of Odc⁺/⁻ mice versus those in Odc⁻/⁻ animals (Fig. 7C).

Discussion

The findings presented herein establish critical roles for macrophage ODC in colonic inflammation and associated colon tumorigenesis. Specifically, mice with myeloid-specific knockout of Odc have more robust cytokine and chemokine responses, yet are protected in terms of clinical parameters and histologic injury in the DSS injury-repair model of colitis. These suggest that macrophage ODC impairs responses to the injury induced by DSS, and suggest that increased macrophage ODC expression in UC may contribute to epithelial cell injury. In contrast, intestinal epithelial cell knockout of Odc does not result in significant changes in the level of DSS-induced colitis. Thus, polyamines derived from ODC have macrophage-selective effects on colonic injury and repair.

Because polyamines are well known to support cell proliferation, ODC has been a target to limit carcinogenesis (24) and/or to limit tumor progression (25), including in colon cancer prevention (26). Here we found that ODC expression is increased in the colonic macrophages of mice treated with DSS, but is not overexpressed in the epithelium in this model. In colitis tissues, immunofluorescence demonstrated strong double-staining for ODC and macrophages, and positive selection studies showed that macrophages represent the myeloid subpopulation with an increase in Odc expression. These data indicate that macrophages...
Figure 7.
Immunofluorescent detection of ARG1, NOS2, and H3K9ac in colonic tumors from Odc<sup>fl/fl</sup> and Odc<sup>Dmye</sup> mice. A tumor area of the colon of AOM-DSS-treated Odc<sup>fl/fl</sup> and Odc<sup>Dmye</sup> mice was used to determine the levels of ARG1 (A), NOS2 (B), or H3K9ac (C) in CD68<sup>+</sup> cells by immunofluorescence. In each panel, CD68 is depicted in red, the protein of interest in green, and the nuclei in blue. CD68<sup>+</sup>ARG1<sup>+</sup> and CD68<sup>+</sup>NOS2<sup>+</sup> cells are shown in yellow, and the presence of H3K9ac in CD68<sup>+</sup> cells is depicted in bright green/white. In A–C, the box in the low power image denotes the area of the higher power image. Scale bars, 100 μm. In C, the open arrowheads indicate cells that are positive for CD68 and negative for H3K9ac; solid arrowheads, cells that are positive for both CD68 and H3K9ac. Quantification was performed using ImageJ and shown in the right panels. *, P < 0.05 for Odc<sup>Dmye</sup> versus Odc<sup>fl/fl</sup> mice.
are the main source of ODC during colitis, although basal ODC in other immune cells could have a role. ODC enzymatic activity has been shown to be increased in colon tissues from humans with familial or somatic APC mutations (27) as well as in the Apc

mouse model of genetically-driven intestinal tumorigenesis (28). However, to our knowledge, ODC has not been immunolocalized in either human colitis or colon cancer. Using a human TMA, we found that ODC protein levels are increased in colonic macrophages in patients with active UC, and with colitis-associated dysplasia or colon cancer, validating the relevance of our animal studies and suggesting the involvement of macrophage ODC in the pathophysiology of colitis and CAC.

In this study, we show that Odc loss in myeloid cells is associated with increased levels of innate immune mediators, including multiple chemokines and cytokines that are hallmarks of M1 macrophages, as well as the Th1 cytokine IFNγ. These results are consistent with our findings that ODC-derived polyamines inhibit macrophage activation in both H. pylori-induced gastritis and in Citrobacter rodentium-induced colitis (10), such that macrophage ODC suppresses innate immune response to infection, through effects on gene transcription, limiting gastritis and colitis in these models. The data reported herein reveal a more complex scenario, where despite increases in proinflammatory chemokines and cytokines that are manifest with myeloid deletion of Odc, the Odc

animals have improved recovery, better preservation of colon structure, and reduced histologic inflammation and epithelial injury. As epithelial deletion of Odc does not provide benefit, these findings support the concept that activation of innate immune responses in M1 macrophages confers protection from colitis. Consistent with this model, deletion of macrophages in mice worsens experimental colitis (29).

Quite strikingly, the increased mucosal innate immune response in Odc

mice impaired tumorigenesis in the colon. Despite this, there was no reduction in histologic colitis and no difference in M1 gene expression in the nontumor areas of the Odc

versus Odc

mice. This could be due to the long recovery period after the final dose of DSS (28 days), but could also reflect the specificity of the increased M1 response against tumors. The reduction of tumorigenesis in Odc

mice was somewhat unexpected, as the inflammatory response is thought to drive the development of tumors in the AOM-DSS model (20, 30), and disease duration of UC in humans is associated with increased risk for colonic dysplasia and carcinoma (31). Further, macrophage depletion in mice using clodronate-filled liposomes reduces tumor number and burden in the AOM-DSS model (18), whereas macrophage depletion in the DSS model augments colitis (29). Also confounding are findings that Tlr4

mice exhibit less mucosal immune response and less tumor development with AOM-DSS treatment (32), yet signaling through MyD88, the main downstream effector of TLR4, reduces DNA damage and tumors in the same CAC model (33). Similarly, although TNFα has been shown to exhibit anticancer properties (34), mice lacking TNF-Rp55 develop less tumors than wild-type animals with AOM-DSS treatment (35).

Given the apparently paradoxical effects of myeloid Odc loss on CAC, we assessed the phenotype of tumor-associated macrophages. Notably, Odc-deficient macrophages display a robust M1 mRNA and protein response, and the M1 phenotype is known to have antitumor activities (36, 37), principally through the production of NO2-derived nitric oxide (36, 38). Further, M1 cells support the development and the proliferation of Th1 cells that exhibit antitumor activities through the production of IFNγ (39, 40) and inhibit Th2-mediated expression of the genotoxic factor activation-induced cytidine deaminase (41). In accordance with these features, IFNγ expression is increased in the tumors of AOM-DSS–treated Odc

versus Odc

mice. In contrast, the myeloid Odc deficiency does not increase the development of intratumoral M2 macrophages that promote tumorigenesis (42) by suppressing immunity via production of TGFβ (43, 44) and IL10 (45), and by supporting angiogenesis (44, 46). Although reduced clinical colitis with DSS may be relevant, our findings are consistent with a model where M1 polarization of macrophages is critical for the host to combat against carcinogenesis, and where macrophage ODC restricts M1 polarization. Further, increased M1 polarization by targeting myeloid ODC is mechanistically linked in vivo to changes in histone marks in tumors, and is consistent with the concept that the polyamine putrescine regulates chromatin structure and M1 target gene expression as found in infectious colitis (10). The lack of differences in tumor infiltrating T cells further points to a key role for the effect of ODC on macrophages.

In conclusion, the findings that macrophage ODC dampens the innate immune response, and M1-driven antitumoral immunity suggests that targeting macrophage ODC is an attractive approach to prevent development of CAC. Thus, agents that can specifically suppress ODC and/or polyamine availability in macrophages may show benefit in high-risk patients with UC-associated dysplasia or other forms of precancerous lesions, such as intestinal metaplasia associated with Barrett’s esophagus and H. pylori infection, which can also lead to inflammatory carcinogenesis. In summary, ODC not only enhances tumor cell proliferation, but also prevents M1 macrophage-mediated antitumor immunity.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors’ Contributions

Conception and design: K. Singh, A.P. Gobert, K.T. Wilson

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Study supervision: K.T. Wilson

Acknowledgments

We thank Joseph T. Roland for technical assistance with the digital image analysis of the TMA. This work was supported by the NIH grants R01AT004821, R01DK053620, P01CA028842, P01CA116087 (subproject 7515), and R01CA190612 to K.T. Wilson; grant 1I01BX001453 from the Department of Veterans Affairs to K.T. Wilson; the Vanderbilt Ingram Cancer Center Support Grant (P30CA06455) and the Vanderbilt Digestive Research Center (P30DK058404) for mass spectrometry, grant IK2BX002126 from the Department of Veterans Affairs to L.A. Coburn; the Vanderbilt Center for Mucosal Inflammation and Cancer; and the Thomas F. Frist Sr. Endowed Chair to J.L. Cleveland. The generation of the TMA was supported by the NIH grants P30CA068485 and P01CA100442, and by a K08 Career Development Award to K.T. Wilson. This work was also supported by NIH grant R01AT006896 to C. Schneider, a fellowship award from the American Heart Association (16POST27250138) to P.B. Luis, and the Moffitt Cancer Center Support Grant P30CA017029, and by the Cotner-Couch Endowed Chair for Cancer Research to J.L. Cleveland.

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Received January 12, 2018; revised May 2, 2018; accepted March 24, 2018; published first May 31, 2018.

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Cancer Res 2018;78:4303-4315. Published OnlineFirst May 31, 2018.

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