Cancer Research

Mast Cells Infiltrating Inflamed or Transformed Gut Alternatively Sustain Mucosal Healing or Tumor Growth

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Abstract

Mast cells (MC) are immune cells located next to the intestinal epithelium with regulatory function in maintaining the homeostasis of the mucosal barrier. We have investigated MC activities in colon inflammation and cancer in mice either wild-type (WT) or MC-deficient ($Kit^{W\text{-}sh}$) reconstituted or not with bone marrow-derived MCs. Colitis was chemically induced with dextran sodium sulfate (DSS). Tumors were induced by administering azoxymethane (AOM) intraperitoneally before DSS. Following DSS withdrawal, $Kit^{W\text{-}sh}$ mice showed reduced weight gain and impaired tissue repair compared with their WT littermates or $Kit^{W\text{-}sh}$ mice reconstituted with bone marrow-derived MCs. MCs were localized in areas of mucosal healing rather than damaged areas where they degraded IL33, an alarmin released by epithelial cells during tissue damage. $Kit^{W\text{-}sh}$ mice reconstituted with MC deficient for mouse mast cell protease 4 did not restore normal mucosal healing or reduce efficiently inflammation after DSS withdrawal. In contrast with MCs recruited during inflammation-associated wound healing, MCs adjacent to transformed epithelial cells acquired a protumorigenic profile. In AOM- and DSS-treated WT mice, high MC density correlated with high-grade carcinomas. In similarly treated $Kit^{W\text{-}sh}$ mice, tumors were less extended and displayed lower histologic grade. Our results indicate that the interaction of MCs with epithelial cells is dependent on the inflammatory stage, and on the activation of the tissue repair program. Selective targeting of MCs for prevention or treatment of inflammation-associated colon cancer should be timely pondered to allow tissue repair at premalignant stages or to reduce aggressiveness at the tumor stage. *Cancer Res; 75(18); 3760–70. ©2015 AACR.*

Introduction

Mast cells (MC) are c-kit–expressing immune cells localized in mucosal surfaces at the interface between the external and internal environment being the first immune cells responding to exogenous stimuli and allergens (1). MCs participate in several physiologic processes and can be viewed as important players in initiation and regulation of immune reactions that occur in their homing tissues, such as the gut (2, 3).

The gut epithelium is the most exposed to the external environment; accordingly, immune reactivity has to be strictly regulated at this site to allow nutrient assimilation and avoid pathological reactions (4). Exposure to molecules damaging the epithelial barrier, but also genetic predisposition or enhanced immune reactivity, may modify gut homeostasis generating inflammation (5).

During mucosal inflammation, luminal antigens enter the mucosa activating the immune regulatory pathways. IL33, a member of the IL1 family of cytokines, acts as an alarmin after being released by epithelial cells to signal the presence of tissue damage (6). This cytokine and the other mediators produced during inflammation affect the immune system that in turn induces epithelial cell proliferation, helping the resolution of tissue damage (7).

The persistence of an inflamed environment is associated with the development of inflammatory bowel diseases (IBD), which includes Crohn's disease and ulcerative colitis. Chronic and relapsing inflammation occurring in IBD has been classically associated with an increased risk of colorectal cancer (8, 9) and epitomizes the well-accepted link between inflammation and neoplastic transformation (10). However, the presence of an overactive immune response in the gut during IBD, *per se*, does not explain the cause of Crohn's disease and ulcerative colitis in mice and humans (11, 12).

The activity of MCs in colon inflammation has been widely studied in mice, but different results have been obtained depending on the model or on the experimental setting

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chosen for the investigation (13, 14). There is also evidence of a pathogenic role for the mouse mast cell protease (mMCP)-6, the mouse homolog of the human tryptase, in dextran sodium sulfate (DSS)-induced colitis (15), but no conclusive data exist about the actual role of mMCP-6 in colon inflammation. MCs are known to accumulate in the inflamed gut of IBD patients (16–18). Also, an increased number of MCs in tumors correlated with poor prognosis in some studies of outcome in colorectal cancer patients (19, 20) and low MC infiltration correlated with lower overall survival in an earlier study (21).

We have investigated MC role in colon inflammation and transformation, modeling colitis and colorectal cancer in c-kit-mutant MC-deficient $Kit^{W\text{-}sh}$ mice (22). Colitis was chemically induced by administration of DSS in the drinking water of wild-type (WT), $Kit^{W\text{-}sh}$ and $Kit^{W\text{-}sh}$ mice reconstituted with bone marrow-derived MCs (BMMC). DSS ingestion causes damage to the epithelial cell barrier and recapitulates the inflammatory condition of human IBD (23). To further analyze the transition from inflammation to cancer, DSS administration was combined with the injection of azoxymethane (AOM), a carcinogen with tropism for colonic tissue (24). Using these animal models of colon inflammation and transformation, we have characterized an unknown function of MCs in colitis and colorectal cancer pathogenesis. The defective recovery from colitis in $Kit^{W\text{-}sh}$ mice was linked to the persistence of proinflammatory signals headed by IL33, which caused a prolonged alteration of intestinal homeostasis. After the development of colorectal cancer, MC infiltration in the tumor stroma became protumorigenic.

Materials and Methods

Mice and treatments

C57BL/6 WT mice of 4 to 6 weeks of age were purchased from Charles River. C57BL/6 c-kit mutant $Kit^{W\text{-}sh/W\text{-}sh}$ (referred to as $Kit^{W\text{-}sh}$) mice were purchased from The Jackson Laboratory and crossed with C57BL/6J, to obtain congenic C57BL/6-$Kit^{+/+}$ WT littermates used as controls in colitis and carcinogenesis experiments.

Mice were maintained under pathogen-free conditions and housed in filter-top cages. Experiments were approved by the Ethics Committee for Animal Experimentation of the Fondazione IRCCS Istituto Nazionale dei Tumori of Milan according to institutional guidelines. The Italian Ministry of Health (Project Number INT07/2009) approved the use of animals for the induction of experimental colitis and colorectal cancer with DSS and AOM/DSS. Mice were administered 1.5% DSS (molecular weight 40,000–50,000; Affymetrix) in drinking water for 10 days. Recovery from acute inflammation was evaluated 7 days after DSS withdrawal. Monitoring of percent loss of body weight from day 0 was used to follow disease course and clinical signs of disease (hunching, diarrhea, rectal bleeding) were combined in a 6-point scoring system and used to monitor disease course (25).

To induce colonic tumors, mice were injected by intraperitoneal (i.p.) route with 10 mg/kg AOM (Sigma Aldrich) and, one week later, exposed to 1.5% DSS in the drinking water for 7 days (modified from ref. 26). Disease course was monitored twice a week and 3 months later, mice were sacrificed by cervical dislocation after anesthesia.

Histology and IHC

Histologic analyses were carried out on paraffin-embedded tissues. The extent of colon inflammation was determined through a 6-point scoring system based on grade and extension of colitis and glandular dysplasia (modified from ref. 27). MC distribution and frequency in colon were assessed by toluidine blue stain, as previously reported (28). MCs were counted in five nonoverlapping high-power microscopic fields (×400) and results were expressed as means.

Human IBD and colorectal cancer samples were collected from the pathology archives of the Human Pathology Section, Department of Health Sciences (University of Palermo, Palermo, Italy), the Human Pathology Section, Ospedali Riuniti Villa Sofia-Cervello (Palermo), and the archives of the Royal London Hospital with approval from appropriate local ethics committee (REC 13/LO/1271; P/01/023). Samples representative of active ($n = 5$) and inactive ($n = 6$) IBD, dysplasia-associated lesions or masses (DALMs, $n = 5$), IBD-associated colorectal cancer ($n = 9$), and sporadic adenoma ($n = 6$) were selected.

All slides were analyzed under a DM2000 optical microscope (Leica Microsystems), and microphotographs were collected using a DFC320 digital camera (Leica). The extent of the neoplastic areas was measured using a Leica DMD108 digital microscope equipped with digital image analysis software.

Isolation of immune cells infiltrating colon

To analyze immune infiltrate in the gut, colons were dissected from mice and epithelial cells were removed by incubating colons for 1 hour at 37°C in medium supplemented with 5 mmol/L EDTA (29). Colons were further digested with 30 μg/μL collagenase IV (Worthington) for 1 hour then filtered with a 70-nm cell strainer (BD Biosciences). Lamina propria mononuclear cells were collected from the interface of a 40% and 75% Percoll gradient (GE Healthcare Life Sciences).

Bone marrow-derived MC differentiation and reconstitution of $Kit^{W\text{-}sh}$ mice

BMMCs were obtained from bone marrows of 3 to 4 congenic mice. *In vitro* differentiation was caused by adding IL3 (20 ng/mL; Peprotech) in the culture medium (30). After 5 weeks of culture, purity of BMMCs was evaluated as percentage of $Fc\varepsilon RI^+$ and c-kit^+ cells. When purity was more than 90%, 10^7 BMMCs were injected i.p. into 6 weeks old $Kit^{W\text{-}sh}$ mice.

Colon cultures and ELISA on supernatants

To perform ELISA assays on culture supernatants, colons were excised from mice and a 1-cm piece immersed in 1 mL of RPMI supplemented with 10% FBS, penicillin (100 U/mL), streptomycin (100 μg/mL), nonessential amino acids (NEAA), sodium pyruvate (1 μmol/L), and β-mercaptoethanol (2.5 μmol/L). Colons were incubated overnight in a 24-well culture plate at 37°C, 5% CO_2. Supernatants were sampled after 24 hours and ELISA performed with IL22 Ready-SET-Go! and IL33 Ready-SET-Go! Kits according to the manufacturer's instruction (eBioscience). The reaction was stopped with 2N H_2SO_4 and the absorbance was measured at 450 nm.

Statistical analysis

Comparisons between two groups were carried out with the two-tailed unpaired Student *t* test, and Welch correction was applied in the presence of unequal variances. In all tests, a

P value of < 0.05 was considered statistically significant (*, *P* < 0.05; **, *P* < 0.01; ***, *P* <0.005).

Results

Mast cells move in areas of epithelial regeneration during DSS-induced colitis

To investigate the role of MCs in colitis, we induced acute inflammation in WT mice by administering 1.5% DSS in drinking water for 10 days. Colons were dissected at different time points during the acute and recovery phase (day 11–17) and the frequency of c-kit and FcεRI double-positive cells in $CD45^+$ $CD11b^-$ infiltrating cells assessed (Fig. 1A).

During acute inflammation (from day 3 to 10), the percentage of MCs among lamina propria (LP) infiltrating cells was similar to that at day 0. In contrast, during the recovery phase after DSS withdrawal (days 14 and 17), the numbers of MCs increased significantly by approximately 2-fold (Fig. 1B)

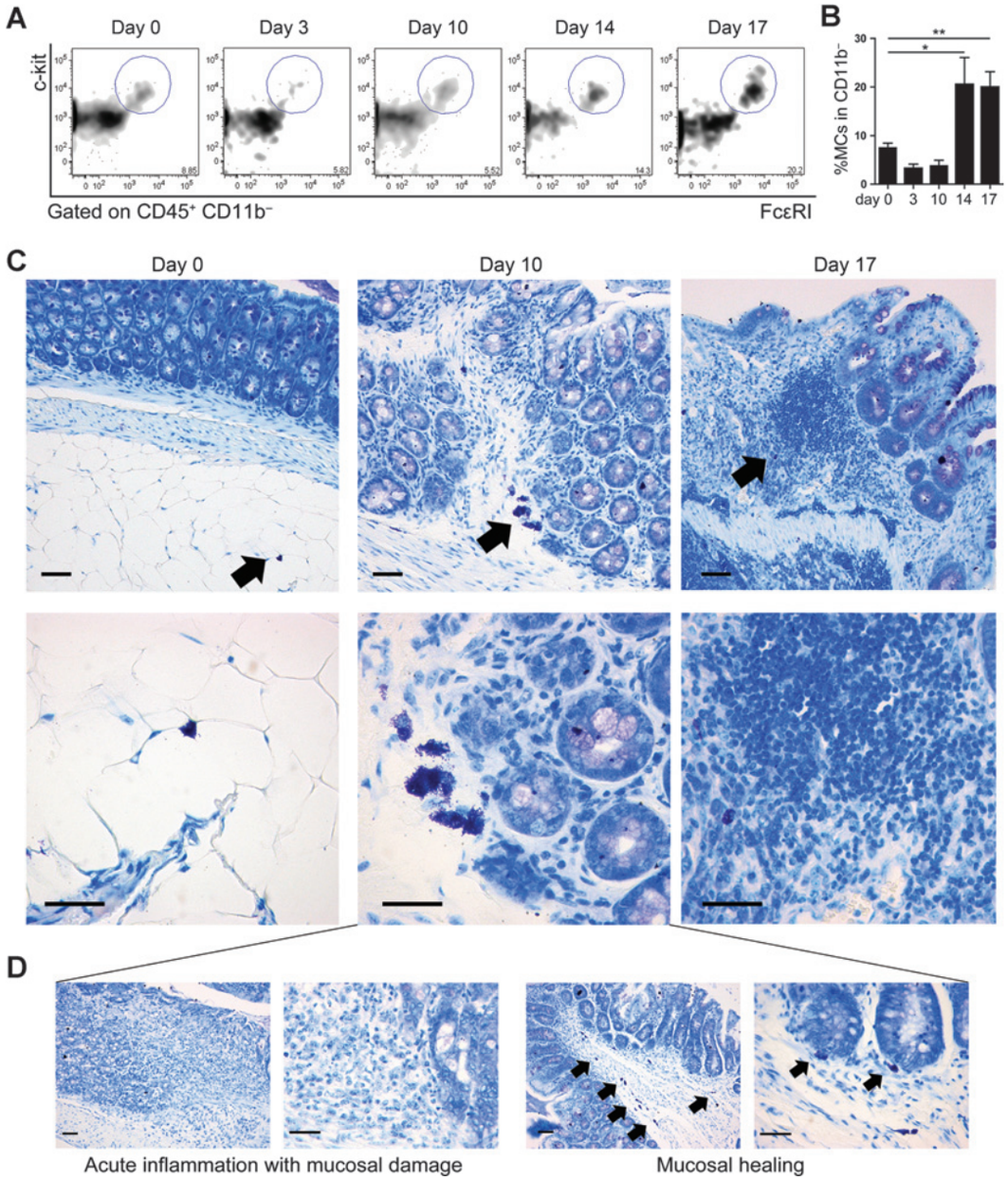

Figure 1.
MC infiltration in colon during DSS-induced colitis. A, MC percentage in LP infiltrating cells was analyzed by flow cytometry. B, mean percentages (±SEM) of LP infiltrating MCs during DSS-induced colitis. Data are pooled from four different experiments (5 mice/group). C, representative toluidine blue stain of colon sections at days 0, 10, and 17. Scale bars, 100 μm, top; 50 μm, bottom. D, representative pictures of MCs in colonic mucosa characterized by acute inflammation (left) or tissue regeneration (right). Scale bars, 100 μm, left; 50 μm, right. Arrows, infiltrating MCs. Student *t* test; *, *P* < 0.05; **, *P* < 0.01.

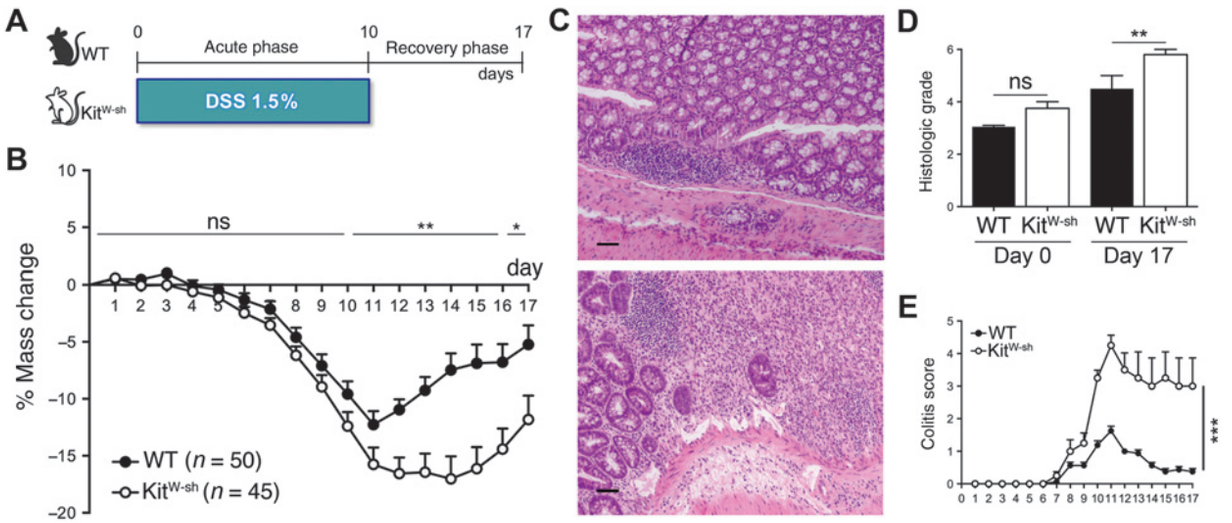

Figure 2.
Course of DSS-induced colitis in wild-type and Kit$^{W\text{-}sh}$ mice. A, experimental scheme of DSS administration. B, percent change in mass of WT and Kit$^{W\text{-}sh}$ mice during the course of colitis. Values are calculated as percent difference of body weight from day 0 and depicted as mean ± SEM. C, representative hematoxylin and eosin sections from WT (top) and Kit$^{W\text{-}sh}$ (bottom) mice colon during recovery from DSS-induced inflammation (day 17). Scale bars, 100 μm. D, histopathological grading (mean ± SEM) of inflammation in WT and Kit$^{W\text{-}sh}$ mice at day 0 and day 17 (5 mice/group from three different experiments). E, scoring of colitis symptoms in WT and Kit$^{W\text{-}sh}$ mice. Results are from three different experiments ($n = 5$ mice/group). Student t test *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.005$; ns, not significant.

implicating MCs in the resolution of inflammation during the repair process.

Analysis of MC distribution within the complex tissue architecture during colitis following toluidine blue staining, a metachromatic marker for MC granules (Fig. 1C) supported the flow cytometry data. Under basal conditions at day 0, MCs were distributed throughout the outer layers of the muscularis propria and serosa close to blood vessels. From day 10, the MCs repositioned themselves from the outer to the inner intestinal layers, closer to the sites of mucosal regeneration. As colitis progressed, MCs moved into the tunica *muscularis* and the mucosa where they contacted the regenerating glands (Fig. 1D). At day 17, MCs were enriched in the healed areas of the mucosa, and in close proximity to lymphoid aggregates within the LP. These results support the hypothesis that MC activity has a role in the resolution of tissue damage induced by DSS exposure.

Kit$^{W\text{-}sh}$ mice show a defective tissue repair activity in DSS-induced colitis

To test our hypothesis that MCs are required for the effective resolution of intestinal inflammation, we compared the response of WT and Kit$^{W\text{-}sh}$ mice during the acute colitic and regeneration phases using our established DSS model. Mice were followed for body weight and disease score until day 17 (Fig. 2A).

DSS induced a significant loss of body weight with a similar progression in both WT and Kit$^{W\text{-}sh}$ mice. Following DSS withdrawal, the rate at which mice recovered their body weight was significantly impaired in Kit$^{W\text{-}sh}$ mice compared with their WT littermates (Fig. 2B). Moreover, a delay in the resolution of inflammation was also confirmed by histologic analyses (Fig. 2C). At day 17 (7 days after DSS withdrawal), the LP of WT mice had already recovered its normal structure, whereas that of Kit$^{W\text{-}sh}$ mice showed severe glandular dysplasia and abundant inflammatory infiltrates. This data has been confirmed scoring histological damage at day 0 and 17 (Fig. 2D). Disease activity index, obtained by analyzing stool consistency, bleeding, and hunching, was also higher in Kit$^{W\text{-}sh}$ mice than in the WT counterpart (Fig. 2E), although colon length shortening, a typical sign of colon inflammation, was similar between the two groups (Supplementary Fig. S1). These data provide evidence that MCs, either directly or through the crosstalk with other cells, are required for the effective resolution of DSS-induced colitis in mice.

BMMC-reconstituted Kit$^{W\text{-}sh}$ mice show a course of colitis similar to the wild-type counterpart

To further confirm MC involvement in the resolution of DSS-induced inflammation, Kit$^{W\text{-}sh}$ mice were reconstituted by i.p. injection of 10^7 BMMCs and, 8 weeks after reconstitution, the same schedule of DSS administration used for WT and Kit$^{W\text{-}sh}$ mice was followed. Body weight loss in reconstituted mice (hereafter Kit$^{W\text{-}sh}$ REC) was comparable with that of the WT counterpart during both the acute and recovery phases of colitis, whereas impaired recovery occurred in Kit$^{W\text{-}sh}$ mice, confirming that MCs are mostly involved in restoring tissue integrity after colitis (Fig. 3A).

This result was confirmed by histologic analyses, which showed that the crypt architecture of Kit$^{W\text{-}sh}$ REC mice was almost normal at day 17 and that the inflammatory infiltrate was more similar to WT than to Kit$^{W\text{-}sh}$ mice at the same time point and experimental condition (Fig. 3B). Furthermore, colons of WT and Kit$^{W\text{-}sh}$ REC mice showed several areas enriched in MCs confirming their increase in number during the recovery phase following DSS withdrawal (Fig. 3C and D).

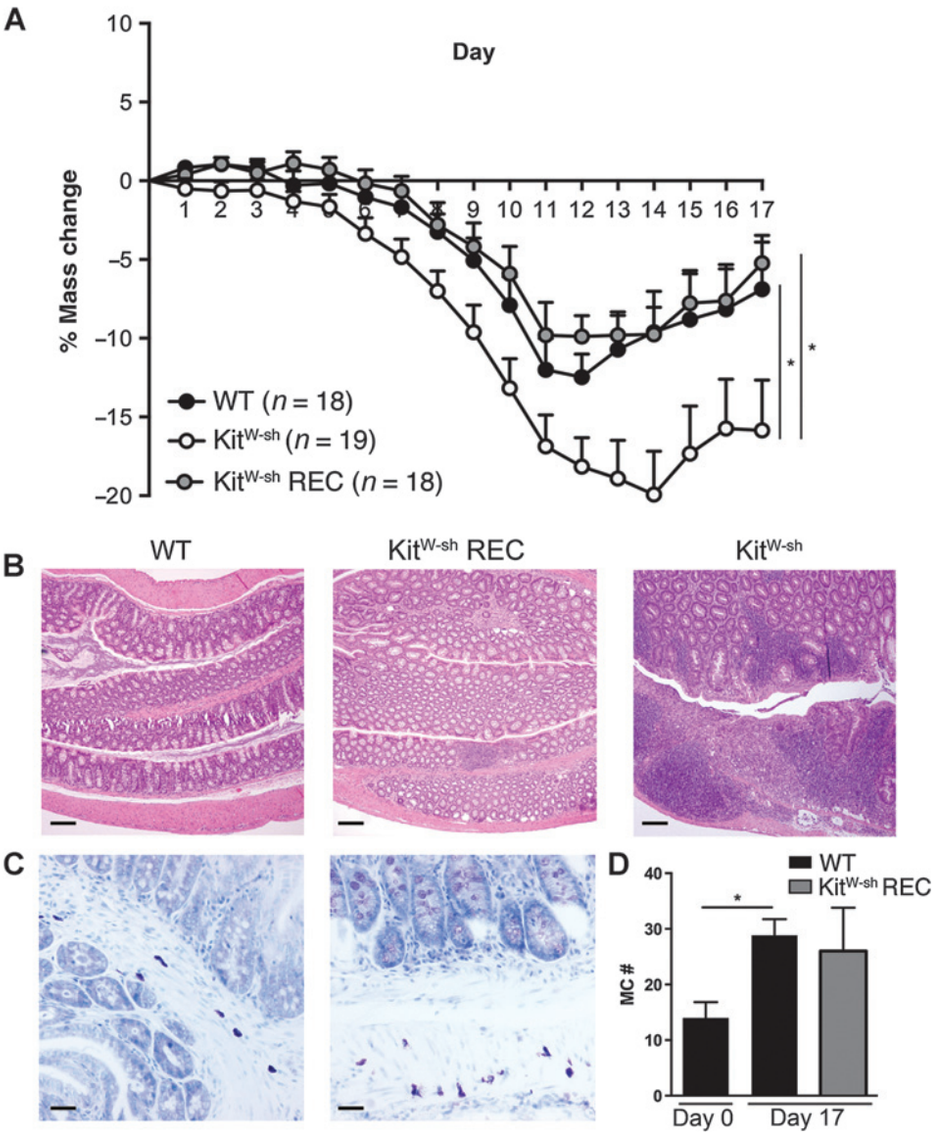

Figure 3.
Course of DSS-induced colitis in wild-type, $Kit^{W\text{-}sh}$, and $Kit^{W\text{-}sh}$-reconstituted mice. A, percent difference of body weight from day 0 in WT, $Kit^{W\text{-}sh}$, and $Kit^{W\text{-}sh}$ mice reconstituted with BMMCs ($Kit^{W\text{-}sh}$ REC). B and C, representative hematoxylin and eosin (B) and toluidine-blue stain (C) of WT, $Kit^{W\text{-}sh}$-reconstituted, and $Kit^{W\text{-}sh}$ mice colons 7 days after DSS withdrawal. Scale bars, 200 μm. D, MC count carried out on toluidine-blue stained colon sections of WT and $Kit^{W\text{-}sh}$-reconstituted mice during recovery from DSS-induced colitis. Values are from two different experiments ($n = 3$ mice/group) and depicted as mean ± SEM. Student *t* test *, $P < 0.05$; ns, not significant.

Mast cell deficiency impact on epithelial cells activity during tissue repair

The alteration in intestinal homeostasis not only may be evaluated through histological damage and persistence of inflammatory infiltration but also assessed analyzing epithelial cell proliferation and the mediators involved in mucosal regeneration.

Thus, we evaluated the proliferation index of colon crypts of $Kit^{W\text{-}sh}$ and WT littermates during the healing process after DSS withdrawal. At day 17, colons from $Kit^{W\text{-}sh}$, but not from WT littermates, showed a significant increase in the percentage of $Ki67^{+}$ epithelial cells (Fig. 4A and D), a result consistent with the continued need for ongoing tissue repair in MC deficient mice.

Epithelial cell proliferation and survival are modulated by IL22, a member of the IL10 family of cytokines, produced by immune cells after various stimuli, among which IL23 is predominant (31). Furthermore, IL22 levels are controlled by IL22-binding protein (IL22bp), a protein that inhibits IL22 binding to its receptor. In homeostatic conditions, IL22 and IL22bp levels are balanced to assure the normal proliferation of epithelial cells. Accordingly, at day 17, we found a higher number of infiltrating cells producing IL23 (Fig. 4B and E) and IL22 (Figure 4C and F) only in colons of $Kit^{W\text{-}sh}$ where inflammation is still not solved. An increase in the expression of *IL22* mRNA in $Kit^{W\text{-}sh}$ relative to WT mice at day 17 was confirmed by qRT-PCR (Supplementary Fig. S2). Furthermore, mRNA levels of *IL22bp*, downregulated during colon inflammation, were reduced in $Kit^{W\text{-}sh}$ mice at day 17 relative to day 0, a finding consistent with increased IL22 signaling in these mice. Altogether, these data strongly support the idea that MCs are involved in epithelial regeneration after mucosal damage.

Mast cells dampen inflammation in an IL33/IL33R-dependent manner

During colitis, damaged epithelial cells release endogenous danger signals. IL33 is relevant (32, 33) because of IL33R

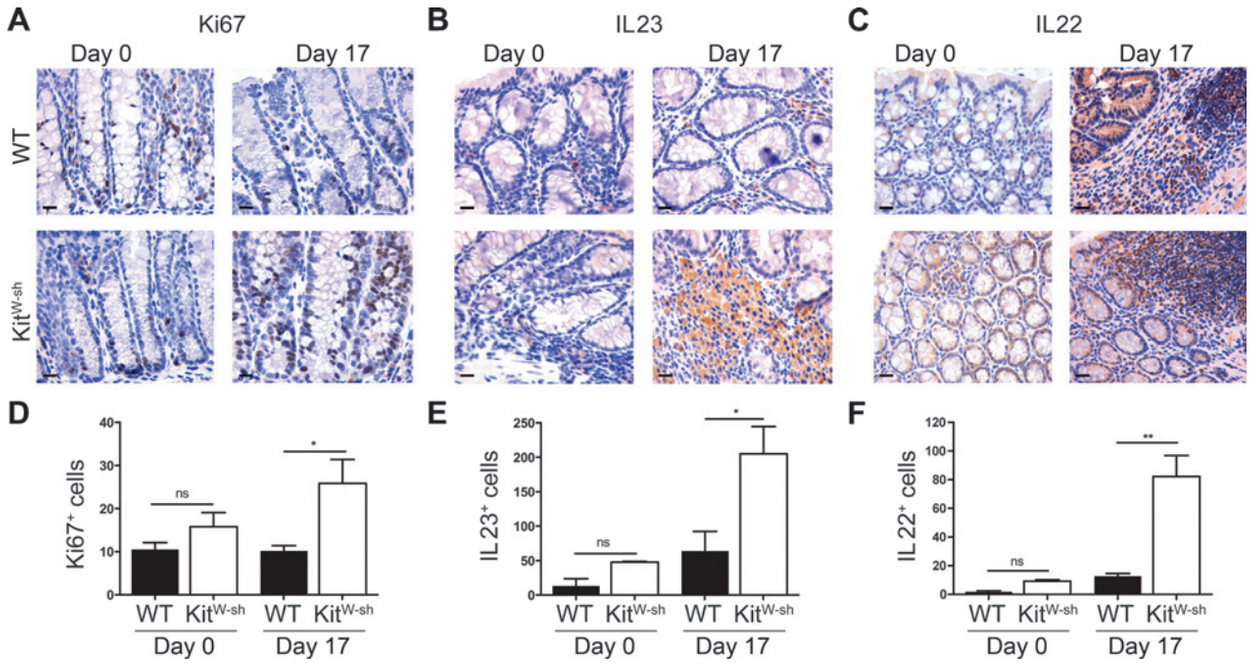

Figure 4.
Activity of colonic epithelial cells in wild-type and Kit$^{W\text{-}sh}$ mice during DSS-induced colitis. Representative stain of Ki67 (A), IL23 (B), and IL22 (C) in WT and Kit$^{W\text{-}sh}$ mice in noninflammatory conditions (day 0) and 7 days after DSS withdrawal. Scale bars, 50 μm. D–F, quantification of Ki67 (D), IL23 (E), and IL22 (F) positive cells in the aforementioned sections. Quantification was carried out in five nonoverlapping high-power microscopic fields ($n = 3$ for each group). Student t test *, $P < 0.05$; **, $P < 0.01$; ns, not significant.

constitutive expression on BMMCs. Hence, we hypothesized that MC can sense and respond to tissue damage through the IL33/IL33R axis.

We used flow cytometry to examine the expression of IL33R by colonic MCs following their expansion in numbers after DSS withdrawal. These analyses showed that less than 5% of MCs express IL33R at the steady state (day 0), whereas 15% of MCs express the receptor at high intensity at day 17 (Fig. 5A and B). At this time point, IL33 was detectable in the supernatant of fresh colonic tissues of WT mice kept *in vitro* overnight but, strikingly, IL33 was significantly higher in the colons of Kit$^{W\text{-}sh}$ mice (Fig. 5C), suggesting that IL33 is either sequestered or destroyed in the presence of MCs. The second hypothesis fits well with MCs known capacity to degrade HSP70, biglycan, HMGB1, and IL33 through the mouse homolog of the human chymase, mMCP-4 (34).

To directly test the role of mMCP-4 in the resolution of DSS-induced colitis, Kit$^{W\text{-}sh}$ mice were reconstituted with 10^7 BMMCs derived from mMCP-4 KO mice or WT mice as control. Mice were then treated with 1.5% DSS for 10 days and monitored as before. In contrast with Kit$^{W\text{-}sh}$ mice reconstituted with WT BMMCs who resolved inflammation and tissue damage after DSS withdrawal, Kit$^{W\text{-}sh}$ mice reconstituted with BMMCs from mMCP-4 KO mice were unable to recover from colitis (Fig. 5D). Critically, the *Mcpt4* gene was highly expressed at time of DSS withdrawal and was maintained at day 17 in colons of WT mice (Fig. 5E). Together, these data suggest that the delayed resolution of inflammation in the Kit$^{W\text{-}sh}$ mice was due to a failure to reduce the IL33 levels that are a key inflammatory response to tissue damage.

Mast cell infiltration in colorectal tumors is associated with high-grade malignancy

Cancer resembles a persistent repair process, a wound that does not heal. To test whether persistent (not yet chronic) inflammation is associated with increased transformation in a context of impaired tissue repair, as observed in Kit$^{W\text{-}sh}$ mice, we injected the carcinogen AOM i.p. one week before treating mice with DSS. After 3 months, we sacrificed mice to determine the extent of tumor development and progression.

The intestinal mucosa of tumor-bearing Kit$^{W\text{-}sh}$ mice was more inflamed in comparison to their WT littermates (Fig. 6A). The fraction of Ki67$^+$ cells increased going from the mucosa to the tumor in both WT and Kit$^{W\text{-}sh}$ mice underling an alteration in epithelial cell proliferation suggestive of active transformation. Nevertheless, Kit$^{W\text{-}sh}$ mice displayed a stronger proliferative hint than their WT counterpart in all the districts analyzed (Fig. 6B) and showed more preneoplastic intestinal polyps, as detected by colon staining with methylene blue (Supplementary Fig. S3A and S3B).

Nevertheless, an analysis of neoplastic areas revealed that WT tumors were more widespread than tumors presenting in Kit$^{W\text{-}sh}$ mice (Fig. 6C). Moreover, tumor grading based on cell differentiation indicated that a much higher percentage of poorly differentiated tumors developed in WT (63.64%) than in Kit$^{W\text{-}sh}$ (30%) mice (Fig. 6D and E). These data suggest that persistent inflammation in Kit$^{W\text{-}sh}$ mice promotes the rate of transformation, but also that the absence of signals from MCs in Kit$^{W\text{-}sh}$ mice attenuates the grade of developing tumors. The latter effect concords with the increase of MCs during adenocarcinoma progression and, accordingly, we found more

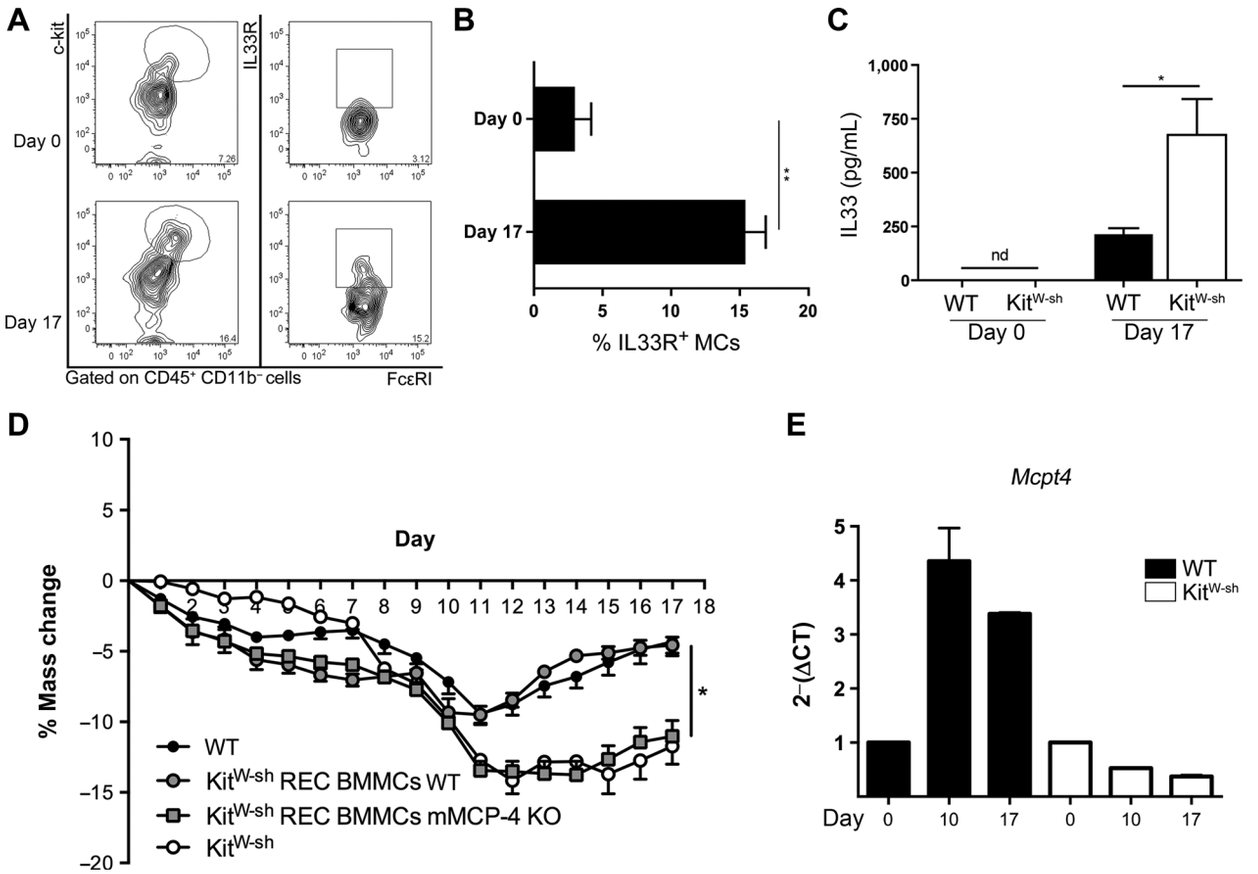

Figure 5.
The IL33/IL33R axis during colon inflammation. A, representative plots showing LP infiltrating MCs and their relative expression of IL33R. B, mean percentages of IL33R expression on colonic MCs at day 0 ($n = 6$) and during resolution from colitis (day 17, $n = 6$). C, evaluation of IL33 levels in colon measured with an ELISA assay on colon culture supernatants. Data are a pool of three different experiments ($n = 5$ mice/group). Values are depicted as mean ± SEM. D, percent mass change of WT ($n = 6$), Kit$^{W\text{-}sh}$ ($n = 7$) and Kit$^{W\text{-}sh}$ mice reconstituted with either WT ($n = 5$) or mMCP-4 KO ($n = 11$) BMMCs. Values are calculated as percent difference of body weight from day 0. E, Mcpt4 expression in colon during colitis progression. Colons were dissected from mice, homogenized, and gene expression levels evaluated with RT-PCR. Student t test *, $P < 0.05$; **, $P < 0.01$.

peri- and intratumoral MCs in less differentiated and more aggressive tumors (Fig. 6F and G).

To confirm these results, AOM and DSS were given to Kit$^{W\text{-}sh}$ mice reconstituted with WT BMMCs expecting that transferred MCs would modify differentiation and grade of developing tumors. Indeed, Kit$^{W\text{-}sh}$ REC mice developed tumors with phenotype resembling their WT counterpart in terms of extension of neoplastic areas (Supplementary Fig. S4A), differentiation, and aggressiveness (Supplementary Fig. S4B). Nevertheless, the proliferation index of tumors from Kit$^{W\text{-}sh}$ REC mice was higher than WT counterpart and similar to that of Kit$^{W\text{-}sh}$ mice, in intra- and peritumoral areas but also in the nearby mucosa (Supplementary Fig. S4C) confirming MC importance in defining tumor grade.

MC numbers are higher in IBD patients following resolution of inflammation compared with active disease and cancer associated with inflammation

To test whether MC behavior in mouse models of colitis and associated-colorectal cancer is similar in the human disease counterpart, we evaluated MC numbers in human samples. MCs were counted in biopsies obtained from active or remitting IBDs and counts were higher in inactive IBD compared with patients with active inflammation (Fig. 7A). This supports the hypothesis that MCs are involved in tissue regeneration following resolution of inflammation as described above in mice. Moreover, DALM and colorectal cancers arising in a context of pre-existing IBD showed a similar significant reduction in MC counts when compared with IBD inactive biopsies (Fig. 7B and C). This is consistent with the hypothesis that persistent inflammation and loss of repair capacity are associated with a reduction in MC numbers; however, MCs are not *per se* necessary for transformation in an inflammatory setting. In contrast, sporadic adenomas (non-IBD) conserve tissue regenerative capacity and a MC density similar to that of IBD in remission. Therefore, adenomas that arise in the absence of inflammation retain their repair capacity and MC numbers.

Discussion

Deciphering the role of MCs in colonic inflammation and tumorigenesis is made complex by MC capacity to mold their function depending on perceived stimuli. Here, we have

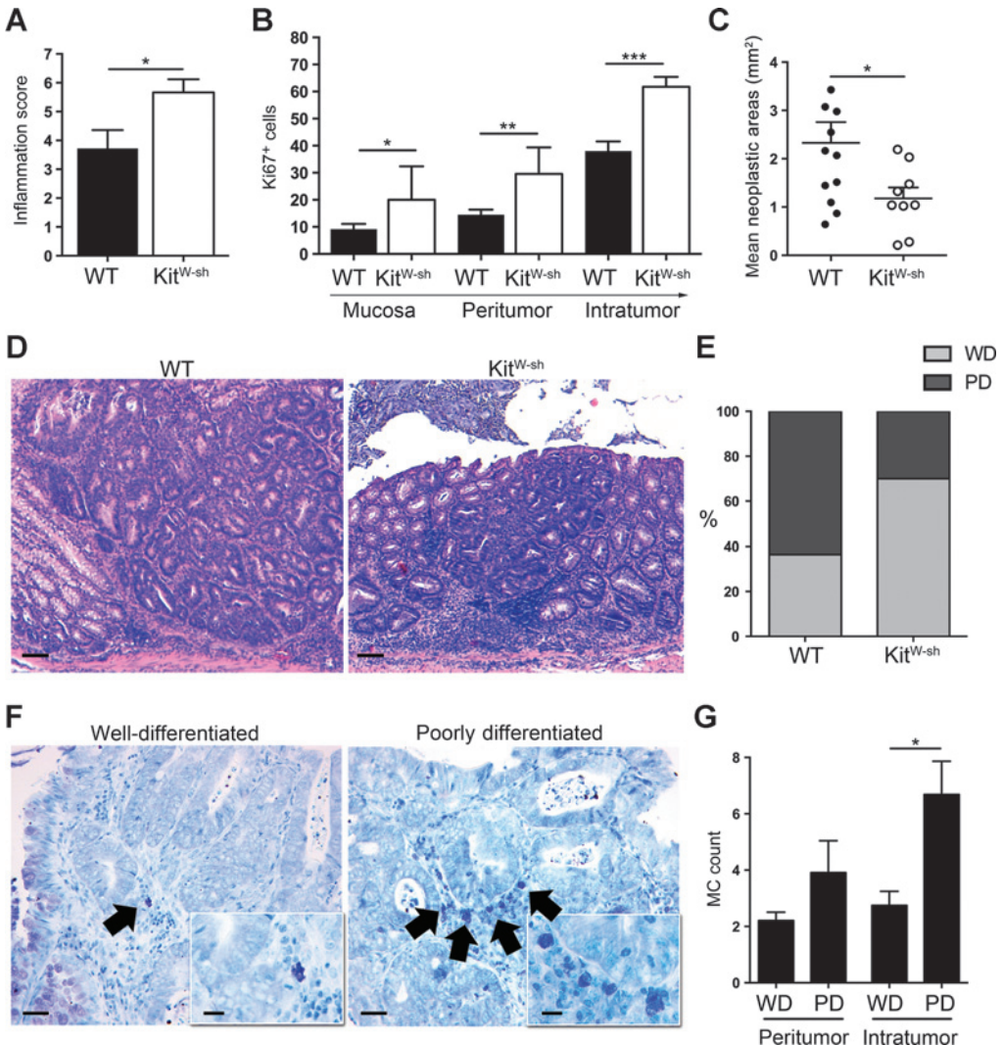

Figure 6.
Colorectal cancer development in wild-type and Kit$^{W\text{-}sh}$ mice. A, scores of inflammation in colon tissues of tumor-bearing mice. The score was calculated combining the grade of immune infiltration, tissue damage, and glandular rarefaction ($n = 5$ mice/group from three different experiments). B, quantification of the percentage of Ki67^{+} cells in colon of WT and Kit$^{W\text{-}sh}$ mice. Ki67^{+} cell percentage was calculated into the tumor, in peritumoral areas, and in the mucosa of five nonoverlapping high-power microscopic fields ($n = 6$ for each group). Values are represented as mean ± SEM. C, mean neoplastic areas in whole colons collected from WT and Kit$^{W\text{-}sh}$ mice. D, representative hematoxylin and eosin staining of a moderately differentiated tumor in a WT mice (left) and an *in situ* adenocarcinoma of a Kit$^{W\text{-}sh}$ mice (right). Scale bars, 200 μm. E, relative percentage of well-differentiated (WD) and poorly differentiated (PD) tumor occurrence in WT and Kit$^{W\text{-}sh}$ mice. F, toluidine blue staining of MC infiltrating well-differentiated or poorly differentiated areas of tumors from WT mice. Inset shows higher magnification. G, peri- and intratumoral-MC count in colorectal tumors. MCs were counted on five high-power fields (×400). Values are depicted as mean ± SEM. Five to seven samples per histologic type were analyzed. Scale bars, 50 μm. Student *t* test *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.05$.

uncovered the role of MCs in repairing DSS-induced colon damage. The increase of MC frequency in the LP of WT mice after DSS withdrawal parallels the delayed recovery of weight loss occurring in Kit$^{W\text{-}sh}$ mice. Our findings propose a novel activity of MCs in colonic epithelial regeneration and add new insights into the role of MCs in intestinal homeostasis. MC accumulation in the inflamed gut was already known (35) albeit in a setting of acute intestinal damage rather than during recovery phase when the regeneration of intestinal crypts is active.

The development of IBD is a multistep process characterized by an unbalanced production of pro- and anti-inflammatory cytokines that progressively perturbs the normal intestinal homeostasis, ultimately leading to the deregulation of epithelial cell proliferation (36, 37). In this context, members of the IL1 cytokines family are chief regulators of innate immunity and inflammation (38). Belonging to this family, IL33 is a *bona fide* alarmin mediating "danger" signals that activate the innate immune responses (39). Indeed, epithelial cell-derived IL33 and its receptor are deregulated in human IBD patients and in mouse models of colon inflammation (40, 41). Here, the relevance of IL33 is further proved by data showing that exogenous IL33 administration is pathogenic during the acute phase of DSS-induced inflammation (42) or that IL33 KO mice are less susceptible to DSS-induced colitis because of reduced granulocytes infiltration (43).

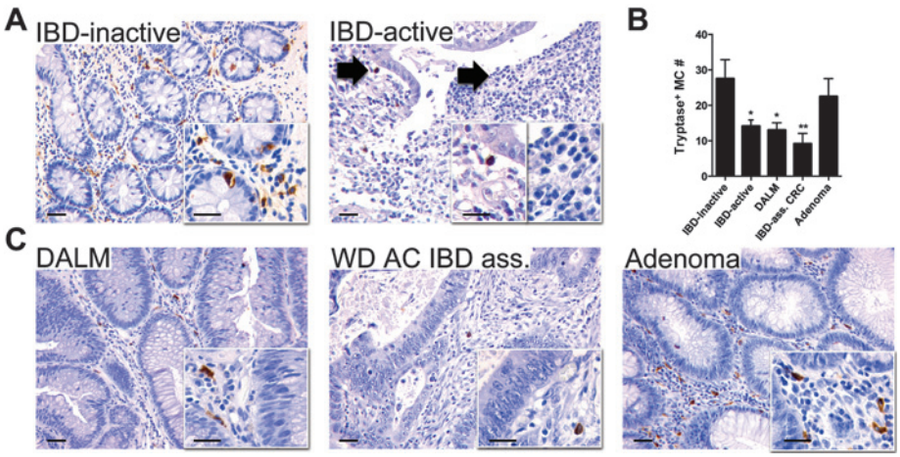

Figure 7.
MC infiltration in human IBD, IBD-associated colorectal cancer, and adenoma. A, IHC staining for MC tryptase of representative samples of active or inactive IBD. In active IBD, insets represent an area of ulcerated mucosa devoid of infiltrating MCs (right) and a MC in the adjacent colonic mucosa (left). B, MC counts carried out on tryptase-stained sections from human biopsies. MCs were counted on five high-power fields (×400) in samples representative of active ($n = 5$) and inactive ($n = 6$) IBD, DALMs ($n = 5$), IBD-associated colorectal cancer ($n = 9$), and sporadic adenoma ($n = 6$). C, IHC staining for MC tryptase of representative biopsies of DALM, IBD associated colorectal cancer (WD AC IBD ass), and a sporadic adenoma. Student t test *, $P < 0.05$; **, $P < 0.001$.

In our model, MCs infiltrating the colon upon DSS withdrawal upregulate IL33R, indicating that IL33 should be active on MCs *in vivo*, when inflammation is resolving or should be resolved. In MC-deficient mice, delayed tissue repair and persistent inflammation are associated with increased levels of IL33 proving again that MCs may be active in the resolution of colitis during the removal of proinflammatory stimuli.

Indeed, MC granules contain a wide range of proteases and mMCP-4, the homolog of the human chymase, has been shown to degrade several alarmins, including IL33, both *in vitro* and *in vivo* (34, 44). Confirmatory evidences in our model came from reconstitution of $Kit^{W\text{-}sh}$ mice with BMMCs from mMCP-4 KO mice: mMCP-4 KO BMMCs were unable to promote mucosal healing after DSS withdrawal, unlike WT BMMCs.

IL22, a member of the IL10 family of cytokines, is protective in the gut: it promotes antimicrobial activity and induces epithelial cell survival and proliferation. The levels of IL22 in colon are controlled by IL22bp, a high-affinity soluble receptor downregulated in the intestine following tissue damage (45). Accordingly, persistent inflammation and incomplete repair of tissue damage occurring in $Kit^{W\text{-}sh}$ mice after DSS withdrawal were associated with higher *Il22* and lower *Il22bp* gene expression than in the WT counterpart.

The role of MCs in resolving colonic inflammation and their capacity to reduce the persistence of inflammatory stimuli suggested that they might be protective against transformation in a setting of inflammation. MCs have been shown to support progression from polyposis to adenocarcinoma in models of chemically induced and oncogene-driven carcinogenesis (13, 46, 47). However, the transfer of the $APC^{Min/+}$ mutation onto the $Kit^{W\text{-}sh}$ background resulted in increased tumorigenesis, highlighting the antitumor activity of MCs in this setting (14).

Our comparison of WT and $Kit^{W\text{-}sh}$ mice during AOM/DSS-induced carcinogenesis showed that the final outcome of MC activity in this context is likely to be dependent on whether the neighboring epithelial cells have the capacity to engage in the healing process or are already transformed (Supplementary Fig. S5). Our results can reconcile apparent controversies regarding the interpretation of MC activity in favor of repair in normal tissues or in promotion of malignancy in transformed cells. Accordingly, tumors with high MC infiltration were less differentiated and more aggressive, whereas mice lacking MCs had more tumors of low grade. Questioning whether MCs should be targeted in cancer clearly requires careful consideration: treatment should be given in context, namely at the beginning of transformation and not during early phases of the inflammatory process.

Also, the comparison with the equivalent human disease underlined the paucity of MCs in cases of detrimental inflammation as occurring in active IBD and IBD-associated colorectal cancers. Conversely, MCs infiltrated areas that were mirroring healing areas of the mouse epithelium such as biopsies of remitting IBD and of tumors arising spontaneously. Indeed, the growth of sporadic adenocarcinomas is driven by genetic alterations and inflammation coevolves with tumor-associated modifications of tissues endowed with repair capacity.

In conclusion, in both mice and humans, MCs acquire a different behavior when faced with normal, damaged, or transformed epithelial cells. This needs to be considered when designing more efficacious approaches to MC targeted therapies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

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