

Decreased susceptibility of mast cell-deficient *Kit^W/Kit^{W-v}* mice to the development of 1, 2-dimethylhydrazine-induced intestinal tumors

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Administration of 1,2-dimethylhydrazine (DMH) induces intestinal epithelial tumors in mice. Increased numbers of mast cells have been reported to occur both within and near a variety of different neoplasms, including DMH-induced intestinal tumors. We investigated the role of the tyrosine kinase receptor, c-kit, and mast cells, in this model by administering DMH to *c-kit* mutant mast cell-deficient mice and the congenic normal mice. We attempted to induce colonic tumors by administering DMH (20 mg/kg body weight, s.c., weekly for 20 weeks) to WBB6F1-*Kit*^{+/+} (+/+) wild-type mice, the congenic mast cell-deficient WBB6F1-*Kit*^{W-v} (*W/W*^v) mice and *W/W*^v mice that had been repaired of their mast cell deficiency by adoptive transfer of bone marrow cells derived from the congenic +/+ mice. The susceptibility to the development of DMH-induced colonic tumors, and the numbers of mast cells associated with these tumors, was evaluated. Normal (+/+) mice exhibited significantly higher numbers of mast cells in DMH-induced intestinal tumors than in macroscopically normal colonic mucosa. Treatment with DMH induced development of colonic tumors in 97% of +/+ mice, but in only 32% of the *W/W*^v mice. *W/W*^v mice that had been repaired of their mast cell deficiency by transfer of +/+ bone marrow cells expressed susceptibility to the development of colonic tumors that was similar to that of wild-type mice. These results show that genetic impairment of c-kit function reduces the susceptibility of mice to DMH-induced colonic tumors, and that defects in bone marrow-derived cells in the *W/W*^v mice contribute significantly to this result. Our findings also are consistent with the possibility that mast cells promote the development of DMH-induced colonic epithelial tumors in mice.

Laboratory Investigation (2005) 85, 388–396, advance online publication, 31 January 2005; doi:10.1038/labinvest.3700232

Keywords: adenocarcinoma; mast cells; colonic tumors; mast cell-deficient mice; 1,2-dimethylhydrazine

Mast cells can produce a wide variety of biologically active products, including histamine, serotonin (in murine rodents), heparin and other proteoglycans, many neutral proteases, several products of arachidonic acid metabolism via the 5-lipoxygenase or cyclooxygenase pathways, and a large number of cytokines, chemokines and growth factors.^{1–5} Many of these mast cell-associated products have been shown to influence various aspects of tumor biology, and several types of tumors in both humans (eg, adenocarcinomas of the breast, pancreas, stomach

and lung^{6–9}) and experimental animals (eg, squamous cell carcinoma¹⁰) have been reported to exhibit increased numbers of mast cells; however, the net effect of mast cells on the development and progression of specific tumors has been difficult to assess.^{11–16} This, in part, probably reflects the complexity of the underlying biology: different mast cell-associated products may have positive or negative effects on different aspects of tumorigenesis or tumor progression.

Several groups have used genetically mast cell-deficient mice (usually, WBB6F1-*Kit*^W/*Kit*^{W-v} [*W/W*^v] mice) and the congenic normal WBB6F1-*Kit*^{+/+} [+/+] mice to search for specific effects of mast cells on the development or progression of particular experimental tumors. Using this approach, some groups have reported an increased susceptibility to tumors in mast cell-deficient mice in certain model systems,^{17–19} while others reported that mast

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Received 24 June 2004; revised 15 November 2004; accepted 16 November 2004; published online 31 January 2005

cell-deficient mice exhibited delayed tumor-associated angiogenesis and/or reduced tumor size and metastasis compared to the corresponding wild-type mice.^{10,20,21}

One explanation of these apparently discordant findings is that the net contribution of mast cells to different aspects of tumor biology *in vivo* can be quite variable, and may critically depend on the model system investigated. In the present study, we used *W/W^v* and congenic wild-type (+/+) mice in an attempt to assess whether mast cells might contribute to the development of 1,2-dimethylhydrazine (DMH)-induced colonic epithelial tumors. We selected this model system because: (1) many colorectal carcinomas in humans are thought to arise as a result of the adenoma–carcinoma sequence;²² (2) the colon carcinogen DMH can induce the development of colonic adenomas in mice, with subsequent development of dysplasia and, finally, colonic adenocarcinomas;^{23–25} and (3) increased numbers of mast cells have been reported in both DMH-induced preinvasive colonic adenocarcinomas in mice²⁶ and DMH-induced colonic adenocarcinomas in rats.²⁷ Moreover, no previous report used genetically mast cell-deficient mice to investigate the potential contribution of mast cells to the development of intestinal tumors, whether induced by DMH or reflecting other pathogenetic pathways of tumor development.

We found that WBB6F1-+/+ mice were significantly more susceptible to the development of DMH-induced colonic epithelial neoplasms than were the congenic *c-kit* mutant WBB6F1-*W/W^v* mast cell-deficient mice. In addition, *W/W^v* mice that had received i.v. injection of bone marrow cells from the congenic +/+ mice (to repair the mast cell deficiency of the *W/W^v* recipients) exhibited a susceptibility to the development of DMH-induced colonic tumors that was similar to that of the wild-type mice. These findings show that genetic impairment of *c-kit* function reduces the susceptibility of mice to DMH-induced colonic tumors, and prove that defects in bone marrow-derived cells in the *W/W^v* mice contribute significantly to this result. Our findings also are consistent with the possibility that mast cells contribute to the development of DMH-induced colonic epithelial neoplasms in mice.

Materials and methods

Mice

Female 5–7-week-old C57BL6/J mice ($n=64$), genetically mast cell-deficient WBB6F1-*Kit^W/Kit^{W/v}* (*W/W^v*, $n=28$) mice and the congenic normal WBB6F1-*Kit⁺/Kit⁺* (+/+, $n=30$) mice, were purchased from The Jackson Laboratory (Bar Harbor, ME, USA). Mice were kept in community cages at light periods of 12 h, and were fed water and mouse chow *ad libitum*. *Citrobacter rodentium* infection was excluded by regular monitoring of the mouse

colonies in the Stanford Laboratory Animal Facility. The *Helicobacter* status of the colonies has not routinely been monitored. All animal care and experimentation was conducted in accord with current National Institutes of Health and Stanford University Institutional Animal Care and Use Committee guidelines. All experiments were performed with age-matched female mice.

Bone Marrow Transplantation

To repair their mast cell deficiency, some *W/W^v* mice (female, 5–7 weeks old, $n=6$, Table 1) received an i.v. injection of 2×10^7 freshly isolated +/+ mouse bone marrow cells. Briefly, bone marrow cells were flushed from the femurs of +/+ mice with cold Dulbeccó's minimal essential medium (DMEM, Gibco BRL, Grand Island, NE, USA). Viable cells were counted with trypan blue exclusion and 2×10^7 cells were resuspended in 200 μ l of DMEM and injected via a tail vein. Control animals received 200 μ l of DMEM without cells.

Induction of Colonic Tumors with DMH

Mice received weekly s.c. injections of either DMH, 20 mg/kg body weight, in NaH₂CO₃-buffered EDTA, 1 mM (pH 6.5), or the vehicle alone (only C57BL/6J, $n=13$), for 20 consecutive weeks. Treatment was started at the age of 5–7 weeks. At 30 weeks after the beginning of treatment, mice were killed by CO₂ inhalation. The peritoneal cavity was opened, the colon removed, the colonic mucosa was exposed with a longitudinal incision and the colonic mucosa was investigated under a stereomicroscope at a magnification of $\times 5$ –30. Tumor occurrence and tumor size (observed with a stereomicroscope and measured with a ruler) were documented. Tissue was harvested and fixed in either Carnoy's fixative (ethanol 60%, chloroform 30%, acetic acid 10%), Karnovsky's fixative (paraformaldehyde 2%, glutaraldehyde 2.5%, CaCl₂ 0.025%, cacodylate buffer 0.1 M) or 4% paraformaldehyde in phosphate-buffered saline (PBS).

Histology and Histochemistry

For quantification of colonic mast cells, 5 μ m sections from Carnoy's-fixed tissues were stained with alcian blue/eosin or toluidine blue and examined under light microscopy at a $\times 400$ or $\times 1000$ magnification. To assess mast cell degranulation, 0.5 μ m Epon-embedded sections of Karnovsky's fixed tissues were stained with Giemsa and examined under light microscopy at $\times 1000$ magnification. To assess whether mast cells contained significant amounts of heparin, 4 μ m Carnoy's-fixed sections were deparaffinized, rinsed in distilled water acidified with 1% citric acid to pH 4.0,

Table 1 Incidence and size of DMH-induced colonic tumors in mice of different genotypes

Mice	Treatment	n	Mice with tumors	P-value	Tumors per mouse	Maximal tumor size (in mm)		
						<1	1-3	>3
C57BL/6J	DMH	64	37 (59%)	<i>P</i> < 0.0001 vs vehicle	0.8 ± 0.1 (range 0-6)	11/37 (30%)	14/37 (38%)	12/37 (32%)
C57BL/6J	Vehicle	13	0 (0%)	<i>P</i> < 0.0001 vs WBB6F1-+/+	0	0 (0%)	0 (0%)	0 (0%)
WBB6F1-+/+	DMH	30	29 (97%)	<i>P</i> < 0.0001 vs WBB6F1-Kit ^W /Kit ^{W-v}	3.5 ± 0.5 (range 0-12)	2/29 (7%)	7/29 (24%)	20/29 (69%)
WBB6F1-Kit ^W /Kit ^{W-v}	DMH	22	7 (32%)	<i>P</i> < 0.0001 vs WBB6F1-+/+	0.5 ± 0.2 (range 0-2)	3/7 (43%)	2/7 (29%)	2/7 (29%)
WBB6F1-Kit ^W /Kit ^{W-v} +BM ^a	DMH	6	6 (100%)	<i>P</i> > 0.05 vs WBB6F1-+/+	2.2 ± 0.4 (range 1-3)	1/6 (17%)	3/6 (50%)	2/6 (33%)
				<i>P</i> < 0.0001 vs WBB6F1-Kit ^W /Kit ^{W-v}				

^aTo repair their mast cell deficiency, these mice received 2.0 x 10⁷ femoral bone marrow (BM) cells from the congenic +/+ mice at 5-7 weeks of age, before the start of DMH injections (see Materials and methods).

stained for 10 s with 0.02% berberine sulfate solution acidified with 1% citric acid to pH 2.5.^{28,29} Slides were briefly rinsed in distilled water (pH 4.0), excess fluid was removed and slides were mounted with glycerol and examined immediately with a fluorescence microscope. Berberine sulfate binds to heparin in mast cell cytoplasmic granules, and the berberine sulfate-positive cells exhibit a bright yellow fluorescence, in a cytoplasmic pattern, when examined under a fluorescent microscope.²⁸ Carnoy's-fixed 4 μm sections of skin from C57BL/6J mice served as positive control for berberine sulfate-positive mast cells.

Assessment of Colonic Epithelial Cell Proliferation

Age- and gender-matched +/+ and W/W^v mice received either DMH (20 mg/kg of body weight in NaH₂CO₃-buffered EDTA, 1mM, pH 6.5). or the vehicle alone, s.c., and were killed 6 h after the injection. At 1 h before killing, mice received i.p. injections of 30 μCi [³H]thymidine (Perkin Elmer Life Science, Boston, MA, USA). After killing the mice by inhalation of CO₂, an ~1 cm long piece of the descending colon was removed. Adjacent tissue was carefully removed and the tissue sample was weighed on a scale. The samples were incubated overnight at 37°C in 500 μl trypsin-EDTA. The samples were then homogenized and transferred to scintillation fluid, mixed well and counted in a beta-counter. The ratio of cpm/mg tissue was calculated.

Statistical Analysis

Data obtained from the different experiments were analyzed using the software Statview Version 5.0. 1 for Macintosh and PC. The incidence of tumor development in various groups was compared using χ²-test. The numbers of adenomas and mast cell numbers/mm² of tissue were compared using the unpaired Student's *t*-test or Mann-Whitney *U*-test. A *P*-value below 0.05 was considered to be significant. Unless otherwise specified, all data are presented as mean ± s.e.m. or mean + s.e.m.

Results

DMH-Induced Colonic Tumors were Associated with Increased Numbers of Mast Cells

Histological examination revealed significantly increased numbers of mast cells at sites of tumor development compared to normal appearing control tissue (Figure 1a-e). Mast cells occurred mainly in the vascularized areas of the tumors, often in close proximity to blood vessels, although occasional mast cells were found in the epithelial mucosa of the tumors. Epon-embedded, 1-μm-thick, Giemsa-stained sections (Figure 1d) showed that most mast cells exhibited small dark cytoplasmic granules, as

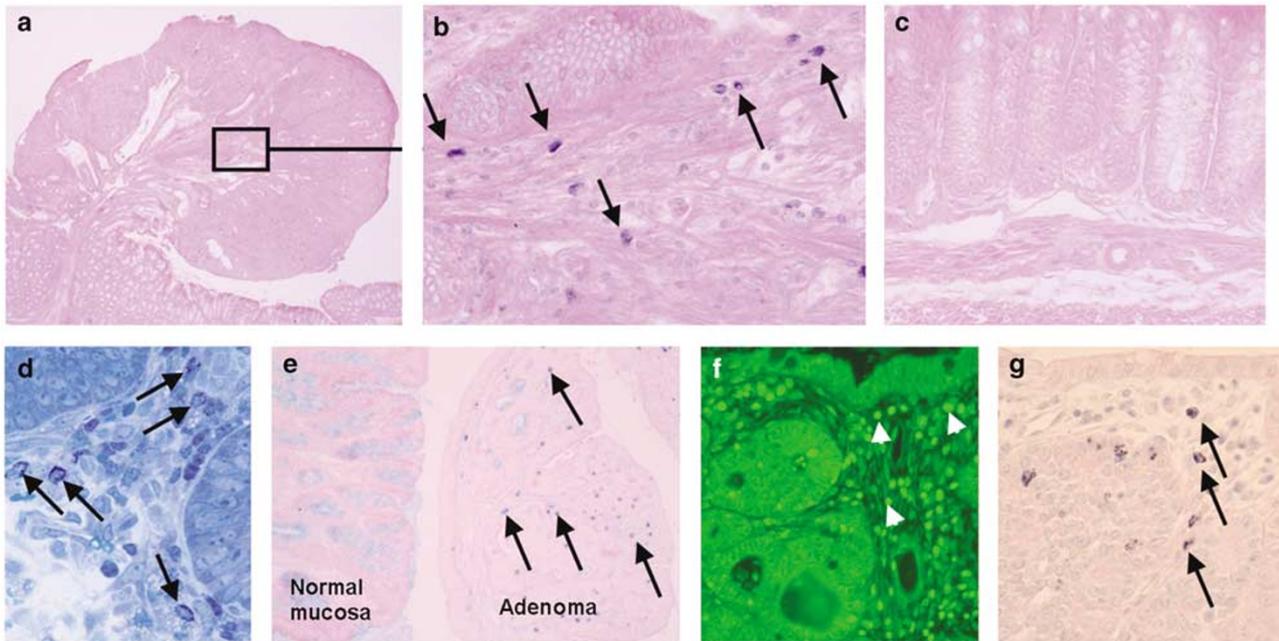


Figure 1 DMH-induced colonic tumors contain increased numbers of mast cells. Photomicrographs of toluidine blue- (a–c, g), alcian blue/eosin- (e), or berberine sulfate- (f) stained, Carnoy’s-fixed, paraffin-embedded 4 μ m sections, and a Giemsa-stained (d) Karnovsky’s fixed, Epon-embedded 1 μ m section: $\times 4$ (a), $\times 40$ (b, c, e–g) and $\times 100$ (d) original magnifications. (b) A magnified image of the tumor shown in (a), reveals increased numbers of mast cells in the tumor stroma (purple-stained cells in b, some of them indicated by arrows). Few mast cells were observed in normal-appearing colonic mucosa (eg, none are evident in the section shown in c, $\times 40$). (d) A Giemsa-stained section of a DMH-induced tumor. No significant histological evidence of mast cell degranulation is evident. (e) An alcian blue-stained section of a colon tumor and adjacent normal colon mucosa. The glandular mucus in the normal mucosa appears light blue. However, many dark blue-stained mast cells (some of them indicated by arrows) can be detected only in the tumor. (f) No berberine sulfate-positive mast cells are present in this section; the yellow-colored structures that stain weakly fluorescent with berberine sulfate are nuclei (some of them indicated with arrowheads). (g) A toluidine blue-stained section adjacent to that shown in (f) reveals the presence of several mast cells (some of them indicated by arrows).

is usually detected in nondegranulated mast cells. Most of the mast cells in the nine adenomas examined after staining with berberine sulfate did not detectably bind berberine sulfate (Figure 1f). A lack of reactivity for berberine sulfate is characteristic of mast cells of the so-called ‘mucosal’ type. In one of the nine adenomas studied, a few of the mast cells exhibited berberine sulfate-positive cytoplasmic granules (not shown).

Quantitative assessment confirmed increased numbers of mast cells in colonic tumors (147 ± 25 mast cells/ mm^2) in comparison to levels in the normal mucosa of DMH-treated mice (8 ± 0.3 mast cells/ mm^2 , $P < 0.0001$) (Figure 2). Moreover, there was a positive correlation between tumor size and the number of mast cells/ mm^2 of tissue in the tumors of DMH-treated WBB6F1-+/+ mice (Figure 3).

Almost all of the WBB6F1-+/+ mice treated with DMH developed intestinal tumors (97%) (Table 1), with a mean of 3.5 ± 0.5 tumors per mouse. Histological sampling of the epithelial tumors indicated that none of the WBB6F1-+/+ mice had developed adenocarcinomas that exhibited invasion through the muscularis mucosae, and none of the mice exhibited gross evidence of metastatic disease. However, several of the adenomatous polyps ex-

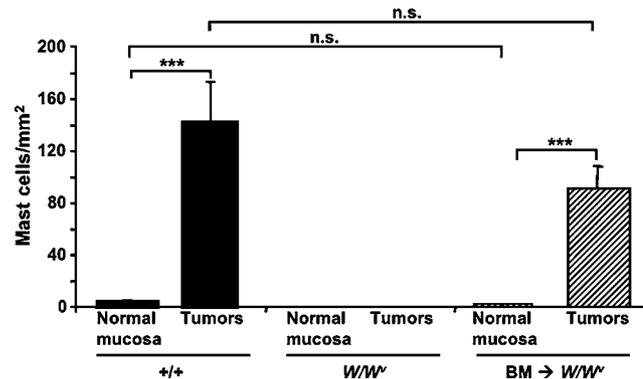


Figure 2 Mast cell numbers are significantly increased in colonic tumors. Mast cells were counted in alcian blue-stained sections of normal-appearing colonic mucosa and in the colon tumors of DMH-treated WBB6F1-+/+ mice (normal mucosa, $n = 15$; tumors, $n = 14$), in mast cell-deficient *W/W^v* mice (normal mucosa, $n = 7$; tumors, $n = 7$) and in *W/W^v* mice that had been repaired of their mast cell deficiency by whole bone marrow transplantation (BM→*W/W^v* mice) at 5–7 weeks of age (normal mucosa, $n = 6$; tumors, $n = 13$). Data were analyzed using the unpaired Student’s *t*-test ($***P < 0.005$).

hibited areas of slight to severe atypia, and in a few cases the lesions had areas of intraepithelial adenocarcinoma. These findings are similar to those that

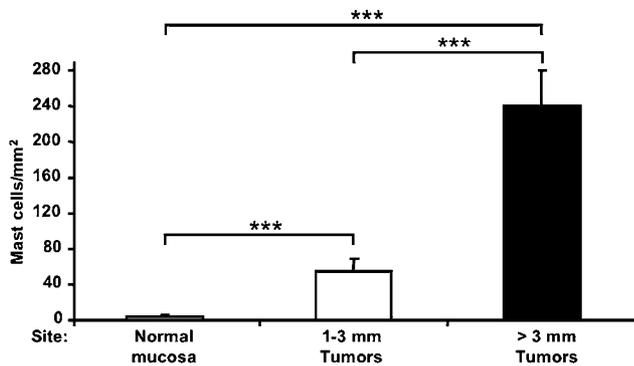


Figure 3 Numbers of mast cells/mm² of tissue increases with tumor size. In DMH-treated WBB6F1-+/+ mice, tumors with a diameter of >3 mm ($n=5$) contained significantly more mast cells than smaller tumors (1–3 mm, $n=6$). Mast cells were counted in alcian blue-stained sections of colon tumors from DMH-treated WBB6F1-+/+ mice. Mast cell numbers in normal-appearing mucosa of DMH-treated WBB6F1-+/+ mice are shown for comparison ($n=14$). Data were analyzed using the unpaired Student's *t*-test (** $P<0.005$).

have been reported in prior studies of DMH-induced epithelial neoplasms in mice.²³

Some mouse strains, such as CF1, BALB/c or SWR/J, have been reported to express a higher susceptibility to DMH-induced tumor development than do WBB6F1-+/+ mice.^{24,30,31} However, when we administered DMH to female C57BL/6 (ie, 'B6') mice (that are semisyngeneic with the WBB6F1 mice used in this study), 59% of the 64 mice tested developed at least one tumor (Table 1), that ranged in size from 1 to 4 mm. The rate of development of tumors in the C57BL/6 mice was significantly less than that in the WBB6F1-+/+ mice ($P<0.0001$). Thus, the rate of tumor development in response to DMH in WBB6F1-+/+ mice, while lower than that reported for mice of the CF1, BALB/c or SWR/J strains, was significantly higher than that in the C57BL/6 strain, that is considered as having 'medium' susceptibility to the tumorigenic effects of this agent.^{24,31} Moreover, mast cell-deficient *W/W^v* mice are only available on the mixed genetic background (WB/REJ × C57BL/6J) and are not available on highly susceptible genetic backgrounds such as CF1. Accordingly, we performed the remainder of our studies entirely with WBB6F1 mice.

Compared to Wild-Type Mice, WBB6F1-*Kit^W/Kit^{W-v}* (*W/W^v*) Mice Developed Fewer Tumors after Treatment with DMH

To investigate the potential importance of *c-kit* and mast cells in DMH-induced tumors, we compared DMH-induced tumor formation in genetically mast cell-deficient, *c-kit* mutant *W/W^v* mice and the congenic wild type (+/+) mice. We found that *W/W^v* mice developed significantly fewer DMH-induced tumors than did wild-type mice (Figure 4). Only seven out of 22 WBB6F1-*W/W^v* mice (32%)

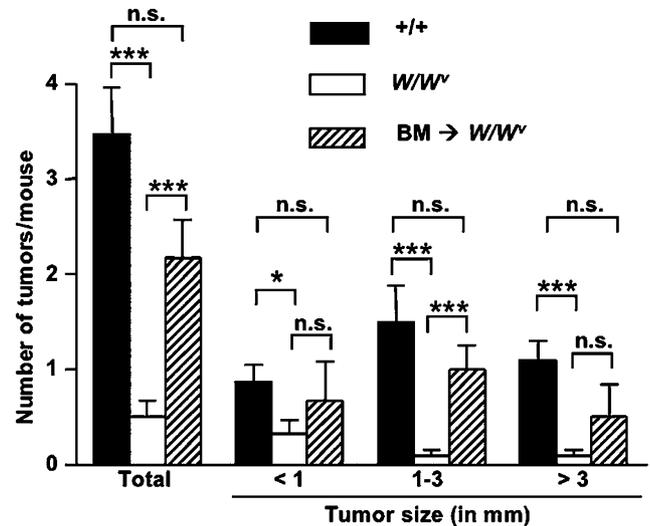


Figure 4 DMH-induced tumor development is significantly impaired in WBB6F1-*W/W^v* mice. Numbers of colon tumors (total, and also shown according to size) 30 weeks after the beginning of weekly s.c. treatment with DMH for 20 weeks in genetically mast cell-deficient *W/W^v* mice ($n=22$), the congenic wild type (+/+) mice ($n=30$) and *W/W^v* mice ($n=6$) that had been repaired of their mast cell deficiency by whole bone marrow transplantation (BM → *W/W^v* mice) at 5–7 weeks of age. Data were pooled from three independent experimental groups that gave similar results, and the data were analyzed using the unpaired Student's *t*-test (* $P<0.05$, ** $P<0.01$ and *** $P<0.005$; n.s. = $P>0.05$).

developed colonic tumors; significantly less than in DMH-treated WBB6F1-+/+ mice (29 out of 30; 97%) ($P<0.0001$) (Table 1). Moreover, the average number of tumors per mouse was dramatically reduced in *W/W^v* mice (0.5 ± 0.2 tumors per mouse) vs the congenic wild-type mice (3.5 ± 0.5 tumors per mouse, Figure 4). As expected, no mast cells were detected histologically in either the normal appearing gut tissue or in any of the tumors that developed in the *W/W^v* mice (Figure 2).

Adoptive Transfer of Wild-Type Bone Marrow Cells to WBB6F1-*Kit^W/Kit^{W-v}* (*W/W^v*) Mice Increased the Recipients' Susceptibility to the Development of DMH-Induced Tumors

To assess the extent to which the differences between the responses of *W/W^v* and +/+ mice represented consequences of the *c-kit* deficiency on mast cells or other hematopoietic lineages in the *W/W^v* mice, we investigated the tumor susceptibility of *W/W^v* mice that had received adoptive transfer of wild-type whole bone marrow cells (+/+ BM → *W/W^v* mice; $n=6$). +/+ BM → *W/W^v* mice exhibited a susceptibility to DMH-induced tumors that was similar to that of the wild-type (+/+) mice, and that was significantly greater than that of the mast cell-deficient *W/W^v* mice ($P<0.0005$) (Figure 4). All of the bone marrow-reconstituted *W/W^v* mice developed intestinal tumors, with a mean of 2.2 ± 0.4

adenomas per mouse. No statistically significant differences were found between the incidence or numbers of tumors in wild-type mice and genetically mast cell-deficient mice that had been repaired of their mast cell deficiency by bone marrow transplantation (Figure 4). Mast cell numbers in the tumors of the bone marrow-reconstituted *W/W^v* mice were similar to the number of mast cells found in the tumors of wild-type animals (Figure 2). Furthermore, mast cell numbers at various anatomical sites (ear skin, stomach, colon) of the *+/+ BM→W/W^v* mice were similar to those in wild-type *WBB6F1-+/+* mice (data not shown).

Histological examination of tumors and the adjacent non-neoplastic colon mucosa in a total of 18 individual specimens from six *c-kit* mutant mast cell-deficient *W/W^v* mice, eight congenic wild-type mice, and two *W/W^v* mice which had received bone marrow cells derived from the congenic wild-type mice (*+/+ BM→W/W^v* mice), also showed variable but generally modest focal inflammatory infiltrates in some areas of the tumors, consisting primarily of lymphocytes with occasional plasma cells and granulocytes. The non-neoplastic colon mucosa adjacent to the tumors generally exhibited either minimal or no inflammation.

DMH Reduces Colonic Epithelial Cell Proliferation *In Vivo* to a Similar Extent in *WBB6F1-+/+* and *W/W^v* Mice

Acute changes that have been associated with DMH exposure include an abrupt reduction in colonic DNA synthesis that is followed by an increasing occurrence of aberrant nuclei in the epithelial layer.³² To assess whether the presence of mast cells or other *c-kit*-related factors might alter the effect of DMH treatment on the proliferation of colonic epithelium, we investigated the acute effects of DMH treatment on DNA synthesis in specimens of colon of *W/W^v* and *+/+* mice. As previously reported, a significantly decreased uptake of tritium-labeled thymidine was detected in DMH-treated *+/+* mice in comparison to vehicle-treated *+/+* mice ($P < 0.05$) (Figure 5).³² The same effect was detected in *W/W^v* mice. Indeed, there were no statistically significant differences between *+/+* and *W/W^v* mice for [³H]thymidine incorporation values for either control (vehicle-treated) or DMH-treated mice (Figure 5). This experiment suggests that the differences in adenoma development after DMH treatment of *W/W^v* mice vs *+/+* control mice probably do not reflect significant differences in the bioavailability of DMH in mice of the two genotypes.

Discussion

Our data clearly demonstrate that *WBB6F1-W/W^v* mice are substantially less susceptible than the

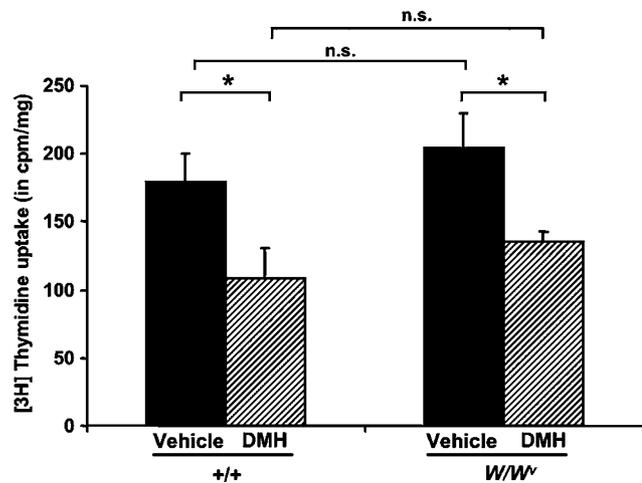


Figure 5 The effect of acute treatment with DMH on colon [³H]thymidine uptake *in vivo* is not altered in *W/W^v* mice. Acute effects of DMH exposure on epithelial cell proliferation in the colon, as reflected by [³H]thymidine uptake in specimens of colon measured 6 h after injection of DMH and 1 h after injection of [³H]thymidine. [³H]thymidine uptake was significantly decreased after DMH exposure in both *W/W^v* mice ($n = 5$ for DMH treatment and $n = 5$ for vehicle treatment) and the congenic normal (*+/+*) mice ($n = 8$ for DMH treatment, $n = 8$ for vehicle treatment). Data were analyzed using the unpaired Student's *t*-test ($*P < 0.05$).

congenic wild type (*WBB6F1-+/+*) mice to the development of colonic epithelial neoplasms in response to DMH treatment in the model system tested. We found that *W/W^v* mice that had been repaired of their mast cell deficiency by adoptive transfer of bone marrow cells from the congenic *+/+* mice had a susceptibility to DMH-induced tumor development that was very similar to that of the wild-type mice. Thus, it is clear that a bone marrow-derived cell type (or types) is/are responsible for the increased susceptibility of wild-type mice to DMH-induced tumor development, as compared to *c-kit* mutant *W/W^v* mice.

However, our findings are less conclusive concerning which specific hematopoietic cell lineages in the *W/W^v* mice are responsible for this effect. In the *+/+* mice or *+/+* bone marrow-reconstituted *W/W^v* mice, the DMH-induced tumors contained large numbers of mast cells, and numbers of mast cells were greater in large tumors than in the smaller tumors. Finally, the tumors that did develop in the genetically mast cell-deficient *W/W^v* mice included a larger proportion of small lesions (<1 mm) than did those that developed in either the *+/+* mice or the *+/+* bone marrow-reconstituted *W/W^v* mice (Table 1, Figure 4).

While all of these findings support the hypothesis that, in this model of carcinogen-induced tumorigenesis, mast cells can contribute to tumor development and/or growth, other possible explanations of the findings cannot formally be ruled out. Reconstitution of *W/W^v* mice by i.v. injection of

+/+ bone marrow cells results in the appearance of mast cells in the colon and multiple other tissues of the *W/W^v* recipients; however, this 'repair' of the mast cell deficiency of the *W/W^v* mice is not selective for the mast cell.³³ Indeed, over time, it is expected that all hematopoietic lineages in the *W/W^v* recipients will eventually be of wild type (+/+) origin.^{34,35} Thus, we cannot rule out the possibility that some additional abnormality of hematopoietic cells in the *W/W^v* mice contributed to the results observed in our studies, in addition to (or even instead of) the lack of mast cells in these mice. While in some anatomical sites, such as the skin, peritoneal cavity and respiratory system, one can achieve *selective* repair of the mast cell deficiency of *W/W^v* mice by the local or i.v. injection of lineage-committed mast cells that have been derived *in vitro* from hematopoietic precursors of +/+ mouse origin, we so far have not been able to use such approaches to achieve the selective adoptive transfer of a mast cell population to the colonic mucosa of *W/W^v* mice.

To screen for possible effects on the metabolism of DMH due to the presence or absence of mast cells and/or other consequences of the loss-of-function mutations in *c-kit* mutant *W/W^v* mice, we analyzed the effect of DMH treatment on cell proliferation in specimens of colon of wild-type and *W/W^v* mice. Such treatment results in a transient reduction in epithelial cell proliferation.³² Wild-type and *W/W^v* mice exhibited very similar DMH-induced reductions in colon cell proliferation, as assessed by measuring the levels of [³H]thymidine incorporation detected in specimens of colon obtained from these mice. This finding suggests that it is unlikely that mast cells had a significant effect on DMH metabolism in this system.

In addition to resulting in a profound mast cell deficiency, a moderate anemia, white coat color and sterility, the markedly reduced *c-kit* function in *W/W^v* mice also results in a virtual lack of interstitial cells of Cajal.^{36–39} Consequently, *W/W^v* mice have abnormal intrinsic electrical activity in the intestine.^{36–39} While effects of abnormalities in intrinsic electrical activity in the intestine on responses to DMH have not been reported, it is possible that such abnormalities may have influenced our results. For example, the abnormality in intestinal motility may increase contact of the feces with the mucosa, thereby promoting tumor development.⁴⁰ By contrast, the lower mechanical forces in the colon might decrease traction on existing tumors. On the other hand, in humans, constipation has not been reported to influence the incidence of colonic tumor development.⁴¹ Whatever the potential effects of the abnormalities of intestinal motility on DMH-induced colon tumor development in *W/W^v* mice, we think that it is unlikely that these effects significantly influenced the results obtained in our study. The transfer of bone marrow cells from +/+ mice should have had no

effect on the deficit in interstitial cells of Cajal in *W/W^v* mice, as these cells are not of hematopoietic origin, yet the injection of +/+ bone marrow cells virtually normalized the response of *W/W^v* mice to the tumor-inducing effects of DMH (Table 1, Figure 4).

If mast cells do contribute to the development of colonic tumors in response to injection of DMH, by what mechanism(s) might they have this effect? One possibility is that proteases released from mast cells, such as tryptases or chymases, mediate proangiogenic effects, as has been proposed in the setting of skin carcinogenesis.¹⁰ Previous work has shown that the administration of certain protease inhibitors to mice can reduce their susceptibility to tumor formation in response to DMH.^{42,43} However, no evidence was presented in those studies showing that these agents were active because of effects on mast cell-derived proteases. Finally, in addition to releasing mediators, mast cells also can take up biologically active molecules from their environment via endocytosis, and thereby limit the biological activity of such products.⁴⁴ This finding raises the possibility that some of the functions of mast cells in tumor biology may be independent of the cells' ability to respond to local activating signals by degranulation and/or other mechanisms of mediator secretion.

In summary, our results show that the mutations affecting *c-kit* in *W/W^v* mice markedly reduce the susceptibility of these mice to the development of DMH-induced colonic tumors. Moreover, our experiments with +/+ bone marrow-reconstituted *W/W^v* mice indicate that effects of *c-kit* on bone marrow-derived cells contribute significantly to the reduced susceptibility of the *c-kit* mutant *W/W^v* mice to DMH-induced colonic tumors. Because transplantation of wild-type bone marrow cells into *W/W^v* mice not only results in the development of mast cells in the recipients, but eventually can result in the entire hematopoietic system of the recipient mice becoming wild type in origin, our data do not prove that the key hematopoietic cell that enhances susceptibility to DMH in this system is the mast cell. Nevertheless, our findings are consistent with the possibility that mast cells can contribute to the susceptibility of mice to DMH-induced colonic epithelial neoplasms.

Acknowledgements

We thank M Liebersbach and Z-S Wang for excellent technical assistance, and Drs Donna Bouley, Sara Michie and Richard Sibley for helping with the review of the histology. This work was supported in part by United States Public Health Service Grants CA 72074, AI 23990 and HL 67674 (to SJG), and by Deutsche Forschungsgemeinschaft Grant WE 2300/1 (to JW).

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