Susceptibility of \textit{Msh2}-deficient Mice to Inflammation-associated Colorectal Tumors$^1$

Maija R. J. Kohonen-Corish,$^2$ Joseph J. Daniel, Hein te Riele, Gary D. Buffinton, and Jane E. Dahlstrom

Department of Medicine, University of Sydney, Sydney, NSW 2006 & Division of Molecular Medicine, John Curtin School of Medical Research, Canberra, ACT 0200, Australia [M. R. J. K.-C.]; Department of Molecular and Clinical Genetics, Royal Prince Alfred Hospital, Sydney, NSW 2050, Australia [J. J. D.]; The Netherlands Cancer Institute, 1066 cx Amsterdam, the Netherlands [H. t. R.]; and The Canberra Clinical School, University of Sydney [G. D. B., J. E. D.] and ACT Pathology [J. E. D.], The Canberra Hospital, Canberra, ACT 2605, Australia

ABSTRACT

Patients with longstanding extensive ulcerative colitis have an increased risk of developing colorectal cancer (CRC). There are significant differences in the early pathogenesis of colitis-associated tumors compared with common CRC, whereas the frequency, degree, and significance of microsatellite instability (MSI) as a marker of mismatch repair deficiency in colitis tumors remain unclear. Here we describe the application of the DSS model of chronic colitis to mice with a defect in the \textit{Msh2} mismatch repair gene to discern these early events. These mice do not develop CRC spontaneously without an external trigger. The aim of this study was to determine the effect of the \textit{Msh2} defect on the frequency and grade of colitis-associated colorectal dysplasia and adenocarcinoma in \textit{Msh2}$^{-/-}$, \textit{Msh2}$^{+/+}$, and wild-type (\textit{Msh2}$^{+/+}$) mice and on the MSI status of the tumors. We show that in mice with chronic colitis, 60% of the \textit{Msh2}$^{-/-}$ and 29% of the wild-type mice developed high-grade dysplasia or adenocarcinoma, but heterozygosity for the \textit{Msh2} defect did not increase tumor susceptibility over wild-type genotype. The largest difference between genotypes was in the frequency of high-grade dysplasia, with 46.7, 8, and 12.5% in \textit{Msh2}$^{+/+}$, \textit{Msh2}$^{-/-}$, and \textit{Msh2}$^{+/+}$ mice, respectively. The \textit{Msh2}$^{-/-}$ mice developed MSI-high tumors, whereas the majority of the \textit{Msh2}$^{+/+}$ and wild-type tumors had no MSI. In the \textit{Msh2}$^{-/-}$ mice, MSI appeared early in non-neoplastic colon tissue, presumably as a result of markedly increased epithelial cell proliferation associated with inflammation. These observations suggest that a homozygous mismatch repair defect predisposes to tumors triggered by chronic inflammation but is not the only factor involved because tumors also developed in the wild-type mice. This model of colitis offers opportunities to characterize the different molecular pathways of carcinogenesis operating in chronic colitis.

INTRODUCTION

Colorectal cancer is one of the most serious and frequent complications of long-standing IBD, particularly in chronic ulcerative colitis, where 10 years after onset, the cumulative risk is 0.5–1% per year in pancolitis patients (1). There are significant differences in the early pathogenesis and molecular alterations of ulcerative colitis-associated tumors as compared with common sporadic colorectal cancer implying that at least the early carcinogenesis is different (2). Dysplasia in sporadic cancers usually presents as a well-defined polyp, whereas dysplasia in ulcerative colitis is diffuse and macroscopically flat. It is well known that failure of cellular mechanisms to detect and repair defects in the DNA sequence is associated with colon carcinogenesis (3, 4). MSI as a marker of DNA mismatch repair deficiency has been described in hereditary and common colorectal cancer, and a standard panel of markers is used to grade tumors to MSI-H, MSI-L, and MSS (5). However, the frequency, degree, and significance of MSI in IBD-associated tumors is unclear. In one study, 9% of 148 colitis tumors were MSI-H, and 11% were MSI-L, with loss of DNA mismatch repair gene \textit{MLH1} expression in half of the MSI-H specimens (6). This frequency distribution resembles that of MSI found in common colorectal cancer (5), whereas another study describes a lower percentage of MSI-H tumors with loss of expression of \textit{MSH2} but normal \textit{MLH1} expression (7). Although the different studies display divergent results, they imply acquired mismatch repair deficiency in a small proportion of tumors triggered by chronic inflammation.

Whereas the pathogenesis of IBD cancer remains poorly understood, there is little dispute that the immune system plays a central role in the initiation and regulation of the tissue injury caused by free radical species (8) and metalloproteases (9) characteristic of the disease. Experimental animal models of IBD have provided new insights to different aspects of the disease in humans (10). Colitis can be induced in mice or rats by supplementing the drinking water with DSS (11, 12). This model shows evidence of free radical species, oxidative injury, and depletion of antioxidants in the inflamed mucosa (13–15) similar to that in human IBD (16, 17). Initially, DSS produces an acute colitis, which becomes chronic after repeated cycles of DSS/water. DSS is not a carcinogen on its own, but dysplasia and adenocarcinoma develop in a small subset of mice with chronic colitis (18).

The \textit{Msh2}-deficient mice have a defect in one of the DNA mismatch repair genes that in humans cause susceptibility to HNPCC (19–21). However, there are some important differences. Mice homozygous for the \textit{Msh2} defect (\textit{Msh2}$^{-/-}$) develop late-onset adenocarcinoma in the small intestine rather than in the colon, the latter of which is the main site of cancer in HNPCC. Mice heterozygous for the \textit{Msh2} defect do not develop spontaneous small intestinal or colon tumors in contrast to heterozygous carriers of the corresponding human mutations. The aim of this study was to examine the effect of chronic inflammation on the frequency of colorectal dysplasia and cancer in \textit{Msh2}-deficient mice and examine the MSI status of the tumors. We show that \textit{Msh2}$^{-/-}$ mice develop a significantly higher frequency of colon tumors than the heterozygous \textit{Msh2}$^{+/+}$ and wild-type \textit{Msh2}$^{+/+}$ mice. The MSI status of the lesions was also different, with the \textit{Msh2}$^{+/+}$ mice developing tumors with MSI, whereas the heterozygous and wild-type mice developed mostly MSS neoplasia. This system offers opportunities to characterize the different molecular pathways of carcinogenesis operating in chronic colitis.

MATERIALS AND METHODS

\textbf{Mouse Breeding.} The \textit{Msh2}$^{+/+}$ mice on the 129/OLA background (20) were crossed with wild-type C57BL/6j to obtain \textit{Msh2}$^{+/+}$ males and females. These were subsequently crossed to produce \textit{Msh2}$^{+/+}$, \textit{Msh2}$^{-/-}$, and \textit{Msh2}$^{+/+}$ litters. The \textit{Msh2} genotype was determined by using a 3-primer PCR from mouse ear punch tissue developed by N. Toft (University of Edinburgh, Molecular Medicine Center, United Kingdom). The primers were P1 5’-CGG CCT TGA GCT AAG TCT ATT AGG; P2 5’-GTT GGG ATT AGA TCT ATT ATA AGG; P3 5’-GGT GGG ATT AGA TCT ATT ATA AGG.

\textit{Received 10/9/01; accepted 12/5/02.}

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

$^1$Supported by the start-up funds provided by the ACT Cancer Society. G. D. B. was supported by the National Health and Medical Research Council.

$^2$To whom correspondence should be addressed, at Cancer Research Program, Garvan Institute of Medical Research, 384 Victoria Street, Darlinghurst, NSW 2010, Australia. Phone: 61 9295 8322; Fax: 61 9295 8321; E-mail: m.corris@garvan.org.au.

The abbreviations used are: IBD, inflammatory bowel disease; MSI, microsatellite instability; MSI-H, microsatellite instability-high; MSI-L, microsatellite instability-low; MSS, microsatellite stable; DSS, dextran sodium sulfate; HNPPC, hereditary nonpolyposus colorectal cancer; TGFBRII, transforming growth factor $\beta$ receptor type II.
TAA TGC CTG CTC T; and P3', 5'-CCA AGA TGA CTG GTC GTA CAT AAG. The length of the PCR product is 164 bp from the wild-type Msh2+ gene and 194 bp from the targeted Msh2− gene. PCR annealing temperature was 60°C. Fragments were visualized on 6% polyacrylamide gels.

**Induction of Chronic Colitis with DSS.** Groups of Msh2+/+, Msh2−/−, and Msh2−/− mice (12–15 week old) were given repeated cycles of 5% DSS in drinking water as described previously to induce chronic colitis (11) followed by distilled drinking water for 2 weeks. The control genotypes received distilled water only. DSS was normally given for 4 days except for 3 days in cycle 2 (males). The females were given an extra 2 days of DSS in the first cycle to boost the onset of colitis symptoms to the same level as the males. The consumption of DSS solution per cage (3–5 mice) was measured throughout the experiment, and the fluid was changed daily. The mice were also weighed daily, and the symptoms of diarrhea and bleeding were recorded individually for each mouse. Symptoms were scored as either absent (0), slight (1), or severe (4) to permit a qualitative, noninvasive assessment of disease severity. If excessive weight loss or other excessive symptoms were observed, mice were sacrificed early. Otherwise, mice were killed, and organs were collected after five or eight cycles of DSS/water. This study was approved by the Animal Experimentation Ethics Committee at the Australian National University.

**Histopathology.** A total of 162 mice (90 females and 72 males) was analyzed for histopathology. After death, the entire small and large intestines (including anal verge) were removed, opened longitudinally, cleaned, examined for abnormalities, and placed in 10% neutral buffered formalin. After 24 h, these were transferred to 70% ethanol until processed for histopathology. The whole colon was embedded into three paraffin blocks (divided into distal, transverse, and proximal sections, approximately five pieces of tissue per block). Multiple sections of each block were examined. The stomach and representative pieces of the small intestine were mounted for analysis. All blocks, including untreated controls, were analyzed by an anatomical pathologist without knowledge of the treatment given. The features assessed included: inflammation (acute or chronic and graded as mild, moderate, or severe) and presence or absence of ulceration, architectural distortion and crypt loss, lymphoid aggregates, paneth cell metaplasia, and epithelial dysplasia or malignancy. Dysplasia was graded as negative, indefinite for low-grade dysplasia and low- and high-grade dysplasia according to the Riddell classification (22). Adenocarcinomas were classified as mucinous if they showed >50% mucinous differentiation. Presence or absence of lymphomas in the large and small intestine was also recorded. Results were scored on pathology sheets, then decoded and arranged according to genotype. All of the blocks that were classified as indefinite dysplasia had additional multiple levels examined and reanalyzed for pathology. Dysplastic and cancerous areas were marked on each slide for subsequent MSI and mutation analysis.

**MSI Analysis.** Areas of dysplasia and adenocarcinoma were microdissected from H&E-stained serial sections. Normal tissue from the stomach or ear punch DNA from the same mouse was used as a control for each tumor. DNA was prepared using the Puregene DNA Isolation Kit (Gentra Systems, Minneapolis, MN). One mononucleotide (urokinase receptor gene, uPAR) and three dinucleotide markers (D1Mit62, D15Mit93, and D17Mit72) were selected from previous studies (20, 23). The primer sequences for the uPAR marker were kindly supplied by Dr W. Edelman, 5'-GCT CAT CTT GGT TCC GTC TG T (forward strand) and 5'-CAT TCG GTT GAA AGC TCT GA (reverse strand). In addition, a T20 repeat in the mouse pro-1 gene (GenBank M209169) was tested using primers 5'-AGC CAT CAA ACT GAT TTT CCA (forward strand) and 5'-ACA AAG GGA GTG CTA GGT CA (reverse strand). This panel of two mononucleotide and three dinucleotide markers was used to determine the degree of MSI following the recommendation for human colorectal cancer (5). Tumors were classified MSI-H if two or more of the five markers showed instability or MSI-L if only one marker was unstable. An additional two markers, D17Mit123 and D17Mit185, were studied in MSI-L and MSS specimens to confirm MSI status. Primer sequences for all dinucleotide markers were obtained from the Whitehead Institute/MIT Center for Genome Research database.4

One primer from each primer pair was synthesized with a 5' fluorescent tag (HEX, FAM, or TET). PCR amplification was performed in a total volume of 20 µl containing 25–100 ng of DNA, 250 µM deoxyribonucleotide triphosphates, 35–55 ng of each primer pair, and 1.2 units of Taq polymerase (Applied Biosystems, Foster City, CA). PCR was carried out at 94°C (30 s), 56°C–60°C (30 s), and 72°C (30 s) for 35 cycles, after an initial denaturation step of 2 min and with a final elongation step of 60 min. PCR was carried out with the GeneAmp PCR system 9600 and 2400 (Applied Biosystems). Fragments were visualized using ABI 310 Genetic Analyzer (Applied Biosystems) capillary electrophoresis. Specimens were scored as having MSI if there was a significant shift in the microsatellite pattern compared with matching normal tissue which produced a consistent pattern of peaks. The highest peak was chosen as the reference. If this fragment amplified less strongly (i.e., produced a lower peak) than at least one of the neighboring fragments in tumor tissue, this was classified as MSI. In addition, extra peaks often appeared that were negligible or not present in normal tissue.

**Analysis of Short Mononucleotide Repeats.** Three short mononucleotide repeats, JH102, JH104 (24), and a A 4 GA 4 repeat from the TGFβRII (25), were analyzed for insertion-deletion mutations in the dysplasia and cancer specimens. The primer sequences for TGFβRII were 5'-CAT TAC TCT GGA GAC GTT TG C (forward strand) and 5'-TCG TTG CAT TCC ATG TTA C (reverse strand). PCR was carried out with fluorescent primers as above, except that the annealing temperature was 56°C with a 7-min final extension. Fragments were visualized using the ABI 310 Genetic Analyzer. The JH104 and the TGFβRII fragments were also sequenced to confirm results obtained from PCR fragment length analysis.

**Statistical Analysis.** The effect of the Msh2 genotype on the frequency of dysplasia and carcinoma was analyzed by χ2 with exact probability (program StatXact; Cytel Corp., Cambridge, MA). The frequencies were subdivided into four categories (normal, low-grade dysplasia, high-grade dysplasia, and adenocarcinoma), and pair-wise comparisons were made between the different genotypes. Daily basal water consumption (mean/mouse) was determined and analyzed during the first two cycles of DSS supplementation by general linear model ANOVA with genotype and sex as categorical variables (program SYSTAT 7.0.1; SPSS, Inc., Chicago, IL).

**RESULTS**

**Induction of Chronic Colitis.** Groups of Msh2+/+, Msh2−/−, and Msh2−/− mice were given repeated cycles of DSS/water, which caused chronic diarrhea and occasional rectal bleeding. The mean daily consumption of DSS-supplemented water did not differ significantly between genotypes (P = 0.39, general linear model ANOVA), but males consumed more than females (P < 0.001) as expected according to their higher body weight. This was compensated by giving the females 1–2 extra days of DSS in the first two cycles. The least-square means were 3.85, 3.78, and 4 ml/day for the three genotypes with least-square means were 3.85, 3.78, and 4 ml/day for the three genotypes.
Colorectal Dysplasia and Cancer. Most (28 of 30) $Msh2^{-/-}$ mice developed some degree of dysplasia or cancer. Almost half (46.7%) of the $Msh2^{-/-}$ mice developed high-grade dysplasia (Table 1 and Fig. 1C), whereas only 12.5% of the $Msh2^{+/+}$ ($P = 0.002$, $\chi^2$) and 8% of the $Msh2^{+/+}$ mice had this lesion ($P = 0.0001$). After eight cycles, the proportion of adenocarcinoma increased in both the $Msh2^{+/+}$ and $Msh^{-/-}$ mice, but the overall frequency of neoplasia was still 35 and 46%, respectively. About 81% of adenocarcinomas showed mucinous differentiation (Fig. 1D), which is also a feature of human ulcerative colitis. Adenocarcinoma was associated with low- or high-grade dysplasia in 6 of 12 $Msh2^{+/+}$ mice, 9 of 13 $Msh2^{+/+}$ mice, and 3 of 4 $Msh2^{-/-}$ mice. Table 1 summarizes the pathology results for each genotype.

Table 1 Percentage of mice that developed dysplasia/cancer in the colon with DSS treatment

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No treatment</th>
<th>DSS</th>
<th>DSS</th>
<th>DSS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Neoplasia</td>
<td>Indef + LGDb</td>
<td>HGD</td>
<td>AdCa</td>
</tr>
<tr>
<td>3–5 cycles of DSS*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$Msh2^{+/+}$</td>
<td>0 (0/10)</td>
<td>25.0 (6/24)</td>
<td>12.5 (3/24)</td>
<td>16.7 (4/24)</td>
</tr>
<tr>
<td>$Msh2^{+/+}$</td>
<td>0 (0/10)</td>
<td>24.0 (6/25)</td>
<td>8.0 (2/25)</td>
<td>8.0 (2/25)</td>
</tr>
<tr>
<td>$Msh2^{-/-}$</td>
<td>0 (0/19)</td>
<td>33.3 (10/30)</td>
<td>46.7 (14/30)</td>
<td>13.3 (4/30)</td>
</tr>
<tr>
<td>8 cycles of DSS c</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$Msh2^{+/+}$</td>
<td>0 (0/28)</td>
<td>8.1 (3/37)</td>
<td>5.4 (2/37)</td>
<td>21.6 (8/37)</td>
</tr>
<tr>
<td>$Msh2^{-/-}$</td>
<td>0 (0/29)</td>
<td>15.2 (7/46)</td>
<td>6.5 (3/46)</td>
<td>23.9 (11/46)</td>
</tr>
</tbody>
</table>

* Effect of the $Msh2^{-/-}$ genotype on dysplasia/carcinoma development is statistically significant after three to five cycles of DSS/water ($Msh2^{-/-}$ vs. $Msh2^{+/+}$, $P = 0.002$, $\chi^2$; $Msh2^{-/-}$ vs. $Msh2^{+/+}$, $P = 0.0001$; $Msh2^{+/+}$ vs. $Msh2^{-/-}$, $P = 0.69$).

b Indef, indefinite; LGD, low grade; HGD, high-grade dysplasia; AdCa, adenocarcinoma. The frequency of indefinite and low-grade dysplasia is combined because of the occasional difficulty in differentiating between the two (22).

c $P = 0.77$. 

Fig. 1. A. Low-power cross-sectional view of normal large bowel. B, low-power cross-sectional view showing transmural inflammation in the large bowel. C, low-power cross-sectional view of high-grade epithelial dysplasia in a tubular adenoma arising in inflamed large bowel. D, mucinous adenocarcinoma arising in inflamed large bowel. All sections are H&E stained; original magnification: ×100; magnification bar measures 200 μm.
group of mice. In the case of multiple lesions found, the histologically most advanced tumor was considered for each mouse.

**Microsatellite Analysis.** Lymphomas and intestinal tumors from the Msh2<sup>−/−</sup> mice are known to display MSI (19–21, 26). We first tested 20 of these spontaneous tumors from the Msh2<sup>−/−</sup> mice with markers D1Mit62, D15Mit93, D17Mit72, uPAR, and pro-1 and observed MSI in all specimens with one or more of the markers. Colorectal dysplasias and carcinomas from 83 DSS-treated mice were then analyzed for MSI (Table 2). Both the inflammation-associated colorectal tumors and the spontaneous intestinal adenocarcinomas from the Msh2<sup>−/−</sup> mice displayed subtle changes from the reference microsatellite patterns (Fig. 2, A and B). The most sensitive indicator of MSI was the T<sub>24</sub> tract in an intron of the uPAR gene. Altogether, 21 of 27 Msh2<sup>−/−</sup> colorectal tumors showed instability in this locus and in at least one other marker (Fig. 2B). Therefore, these tumors were classified MSI-H following the standard criteria for human colorectal cancer (5). Three of the 27 Msh2<sup>−/−</sup> colorectal tumors showed instability in only one marker (MSI-L), and three were MSS. Other areas of the intestinal tract, such as stomach and proximal small intestine in the DSS-treated mice, showed the same patterns as the normal colon in untreated Msh2<sup>−/−</sup> mice. The exceptions included 2 mice whose tumors were MSS and 5 mice whose tumors were MSI-H.

**Mutations in Short Mononucleotide Repeats and the TGFβRII Gene.** To examine whether the MSI observed would also cause deletion-insertion mutation in shorter repeat sequences, an additional three genes were analyzed. The JH102 and JH104 repeats have been reported previously to be good indicators of MSI in tumors of MLH1-deficient mice (24). JH102 is an A<sub>8</sub> repeat in the coding region of the mouse Apc gene. JH104 is located in an intron of the Msh3 DNA mismatch repair gene. No insertion-deletion mutations were found in any of these markers in 10 MSI-H Msh2<sup>−/−</sup> colorectal tumors analyzed. The JH104 marker was also found to be polymorphic, with a T<sub>9</sub> repeat in the C57BL/6J strain and a T<sub>10</sub> repeat in the 129/Ola strain. The A<sub>8</sub>G<sub>4</sub>A<sub>4</sub> repeat in the coding region of the TGFβRII gene corresponds to the A<sub>10</sub> repeat known to be mutated in human MSI-H colorectal cancers (27) but has not been studied previously in intestinal or colorectal tumors in these mice. We could not find any evidence of insertion-deletion mutations of the A<sub>8</sub>G<sub>4</sub>A<sub>4</sub> repeat in colorectal tumor specimens from 21 DSS-treated Msh2<sup>−/−</sup> mice.

**DISCUSSION**

The recognition of DNA mismatch repair genes in hereditary and common colorectal cancer (3, 4) has led to the analysis of the role of these genes in IBD-associated cancer (6, 7). Genetic association studies implicated mismatch repair genes as important susceptibility genes when the frequencies of an MSH2 splice site substitution were compared in two groups of patients with ulcerative colitis, one with high-grade dysplasia and cancer and one without tumors (28). It was noted that this substitution was more common in the patient group with tumors and concluded that a variant of MSH2, which is not normally deleterious, may predispose to cancer in the setting of chronic colitis. A later study (29) was unable to confirm the association of colitis cancer with MSH2. A significant association between Crohn’s disease/ulcerative colitis, certain polymorphic alleles of the MLH1 gene, and a microsatellite marker located in an intron of MLH1 has been reported (30). However, there is no association between HNPPC and an increased risk for IBD.

This study describes the effect of a DNA mismatch repair defect on the development of dysplasia and cancer when triggered by chronic inflammation. We analyzed mice that were either homozygous, heterozygous, or wild type for the Msh2 DNA mismatch repair defect.

### Table 2 Percentage of mice showing MSI in colorectal dysplasia and cancer

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Indef&lt;sup&gt;a&lt;/sup&gt;</th>
<th>LGD</th>
<th>HGD</th>
<th>AdCa</th>
<th>Total no. of mice</th>
<th>MSI-H</th>
<th>MSI-L</th>
<th>MSS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Msh2&lt;sup&gt;+/+&lt;/sup&gt;</td>
<td>2</td>
<td>7</td>
<td>5</td>
<td>12</td>
<td>26</td>
<td>0 (0/26)</td>
<td>11.5 (3/26)</td>
<td>88.5 (23/26)</td>
</tr>
<tr>
<td>Msh2&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>8</td>
<td>5</td>
<td>4</td>
<td>13</td>
<td>30</td>
<td>0 (0/30)</td>
<td>13.3 (4/30)</td>
<td>86.7 (26/30)</td>
</tr>
<tr>
<td>Msh2&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>2</td>
<td>8</td>
<td>14</td>
<td>3</td>
<td>27</td>
<td>77.8 (21/27)</td>
<td>11.1 (3/27)</td>
<td>11.1 (3/27)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Indef, indefinite; LGD, low grade; HGD, high-grade dysplasia; AdCa, adenocarcinoma.

**Fig. 2.** MSI in Msh2<sup>−/−</sup> mice analyzed with ABI 310 Genetic Analyzer. MSI observed in A–C. A, intestinal cancer or lymphoma (untreated Msh2<sup>−/−</sup> mice); B, colorectal tumors (DSS-treated Msh2<sup>−/−</sup> mice); C, Inflamed colon tissue with no dysplasia or cancer (DSS-treated Msh2<sup>−/−</sup> mice); D, normal colon (untreated Msh2<sup>−/−</sup> mice); E, ear punch tissue (DSS-treated Msh2<sup>−/−</sup> mice). Microsatellite patterns D and E were used as the reference to determine MSI. Arrows, the highest peak for each microsatellite pattern indicating a shift in peak heights in A–C. Black peaks, fragments that were either absent or negligible in normal tissue. Numbers on horizontal axis specify fragment sizes in bp.
There was no difference in the severity of inflammation between the three genotypes. Heterozygosity for the Msh2 defect did not increase tumor susceptibility in the setting of chronic inflammation compared with wild type. Heterozygous carriers of a MSH2 gene defect in humans ultimately develop HNPCC from their late teens as the wild-type allele is mutated in colon epithelial cells. It is not known what triggers the somatic mutation in HNPCC, and no data are available from human IB patients who also are MSH2 mutation carriers. The majority of the Msh2+/- and wild-type tumors in our study appeared to develop independently of the mismatch repair system as very little MSI was found.

In contrast to the maximum frequency of ~30% of high-grade dysplasia or adenocarcinoma found in the wild-type or Msh2+/- mice, 60% of the DSS-treated Msh2-/-/- mice developed these tumors. The largest difference was observed in the frequency of high-grade dysplasia, with 12.5, 8, and 46.7% in Msh2+/-, Msh2-/-, and Msh2-/- mice, respectively, whereas the frequency of adenocarcinoma was similar for each genotype. These results suggest an important connection between the DNA mismatch repair proteins and development of dysplasia and contrast a widely held view that mismatch repair defects accelerate carcinogenesis (31, 32). There is increasing evidence that the mismatch repair machinery is not only activated by mismatched nucleotides but by DNA damaging agents (33, 34). It has been suggested that the mismatch repair proteins may signal damage-induced apoptosis through a direct-sensor signaling process (32, 35). Our study suggests that inflammation-induced damage may also be controlled by mismatch repair proteins, such as MSH2. It will be important to determine whether chronic colitis involves a similar apoptotic pathway (32, 33).

We also observed a significant difference in the frequency of MSI, with the majority of Msh2-/- tumors being MSI-H. However, the individual MSI patterns were much more subtle than we have observed in human colorectal cancer specimens using similar methods but no different from the spontaneous intestinal cancers from the same mice. Nevertheless, the tumor MSI patterns were absolutely reproducible and quantifiable. Interestingly, we were not able to detect any instability in short mononucleotide repeats, which have been reported previously to be mutated in tumors from mismatch-repair-deficient mice. These included the A10/GA4 repeat in the coding region of the TGFBR2 gene, as reported to be mutated in Msh2-/- lymphomas (25). The corresponding A10 repeat is also an important mutation target in human MSI-H colorectal cancers (27). This indicates that other target genes may be mutated or abnormally regulated in this model.

In the majority of DSS-treated Msh2-/- mice tested, MSI was also detected in the colon tissue surrounding the tumor, implying that some MSI is already induced by inflammation and precedes dysplasia. Instability at one or more microsatellite loci has also been found in a proportion of human inflamed non-neoplastic lesions in IB patients (28, 29, 36). It has been suggested that this is caused by saturation of normal DNA repair mechanisms in inflamed colon epithelium because of markedly increased cell turnover and proliferation in IB (29). Our results are compatible with the hypothesis that the increased proliferation induces widespread DNA replication errors, which are repaired normally until the DNA repair systems become saturated. As a result of defective mismatch repair already present in the Msh2+/- mice, this is quickly manifested by the appearance of MSI in the inflamed colon tissue. In the wild-type and Msh2+/- mice, chronic inflammation causes the same DNA damage, but the intact mismatch repair system is able to repair more of the damage.

This still leaves a high proportion of MSS dysplasia and cancer that cannot be explained on the basis of temporary saturation of the DNA mismatch repair system and probably includes the majority of human IB cancers. Therefore, other mechanisms will need to be looked at, such as gene silencing by hypermethylation of CpG-rich promoters and injury to DNA bases directly as a result of the inflammatory response. In conclusion, we have described a novel application of a previously well-characterized animal model of chronic colitis. We have shown that by studying colorectal dysplasia and cancers developing in mice that are either homozygous, heterozygous, or wild type for a DNA mismatch repair defect, parallels can be drawn to human chronic colitis-associated cancers. Additional molecular genetic analysis of this model is likely to reveal more insights to the early pathogenesis of dysplasia and the different molecular mechanisms of carcinogenesis operating in IBD cancers.

ACKNOWLEDGMENTS

We thank Jo McIntosh and the Specific Pathogen Free Animal Breeding Unit at the John Curtin School of Medical Research for supplying the mice; Antje Janssen, Kavitha Kugathas, Natasha Tettow, and June Hornby for expert technical assistance; William F. Doe for his encouragement; Richard Fishel for his generous advice; and Ronald J. A. Trent for his valuable support.

REFERENCES


Susceptibility of *Msh2*-deficient Mice to Inflammation-associated Colorectal Tumors


Updated version  Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/62/7/2092

Cited articles  This article cites 35 articles, 13 of which you can access for free at: http://cancerres.aacrjournals.org/content/62/7/2092.full.html#ref-list-1

Citing articles  This article has been cited by 15 HighWire-hosted articles. Access the articles at: /content/62/7/2092.full.html#related-urls

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.