Natural History of Aberrant Crypt Foci
A Surgical Approach

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BACKGROUND: The aberrant crypt focus (ACF) appears to be an important early step in colorectal carcinogenesis. Our objectives were to determine the natural history of ACF in a surgical model. METHODS: The natural history of ACF was followed by marking the lesions in vivo with tattoos. Rats were given four weekly injections of azoxymethane (AOM; 20 mg/kg). One hundred days after the first injection of AOM, rats were anesthetized, and individual aberrant crypt focus was identified by staining with methylene blue. A 3 x 3 mm area, identifying one large (4-8 crypts) ACF was marked with a tattoo dye in each colon. Control animals received saline or AOM injections and were tattooed in areas without ACF. At 200 days, colons were examined for the presence of macroscopic lesions. RESULTS: A total of 54 tumors were found in the study group of 38 animals, and 21 of these were in the transverse and proximal descending colon. The marked areas (all in transverse and proximal descending colon) yielded 6 tumors and 2 ACF, but in 30 instances no abnormality was noted. Probability of observing a tumor in the 3 x 3 mm area of the colon that was identified as containing ACFs was 17 times greater than expected from the observed tumor rate in approximately the same zone (16 vs. 1.7 percent; 95 confidence interval, 10 to 22 and 0.5 to 1.3 percent). Twenty control animals receiving saline or AOM injections and were tattooed in areas without ACF had no tumors. Nine control animals that were carcinogen-treated and tattooed in areas without ACF had no tumors in the marked areas. CONCLUSION: Results thus show regression of many ACF identified early in the carcinogenesis process. Results also support the hypothesis that some ACF are precursor lesions for adenomas and cancers.


Colon cancer is thought to develop as a multistep process. Steps in this process include changes from normal epithelium, through a hyperproliferative state, to clinically observable adenoma, to frank carcinoma. The step that has only recently received attention is the early focal abnormality in the normal mucosa, the aberrant crypt focus (ACF). These foci can be observed on the mucosal surface after staining the colon with methylene blue. They appear as enlarged, slightly elevated and darkly staining crypts. Shortly after a carcinogen exposure, ACF are seen as single isolated abnormal crypts, but with time they appear more frequently in clusters of two or more abnormal crypts per focus.1

It is reasonable to suppose that the ACF fits as an early step in the multistep carcinogenesis process. Focal genetic or epigenetic events in the normal epithelium could lead to focal morphologically abnormal, aberrant crypts. These aberrant crypts could divide slowly in a manner similar to normal crypts to give rise at first to larger foci of aberrant crypts (ACF) and later to adenoma. Although this model appears reasonable, support for it is limited.

The purpose of this study was thus to follow the natural history of ACF. We used the well-established colon carcinogen, azoxymethane (AOM), to induce ACF and then 100 days later surgically opened the colon and identified relatively large (4-8 crypts/focus) ACF in the mucosa using a dissecting microscope. We marked these lesions using tattoo ink and, after closing the colon and incision, followed the animals to the appearance of colonic tumors from 200 days. We anticipated that the tattooed areas (TA) would later contain large ACF, polyps, or cancer and that, perhaps, one-third of these relatively large ACF would have formed macroscopic tumors. This did not turn out to be the case. Most TA identified had only normal colonic epithelium, and frequency of tumors was lower than expected. However, the study did support the hypothesis that some ACF do represent an early step in the development of colon cancer.
METHODS
Animals
Fisher F344/NHSD female rats (Harlen Sprague Dawley Ltd., Indianapolis, IN) weighing 60 g were housed in plastic cages and fed Teklad™ 6 percent Mouse/Rat Diet (Harlen Teklad, Madison, WI) ad libitum except as noted. The protocol was approved by the Ontario Cancer Institute Animal Care Committee.

Initiation and Experimental Design
Four groups of animals were involved.

Group A (n = 10). Group A was given four weekly intraperitoneal injections of AOM (Sigma Chemical Co., St. Louis, MO; 20 mg/kg) and were killed 100 days later to establish number and distribution of ACF at 100 days, the time we chose to mark the ACF. Distribution was given for three segments of the colon of approximately similar areas: 1) right colon; 2) transverse and proximal descending colon, which was the area visualized and examined at operation; 3) rectosigmoid. The transverse and proximal descending colon segment was selected for technical access reasons.

Group B, the Study Group (n = 66). Group B was given four weekly injections of AOM as were those in Group A. One hundred days after the first injection, rats were anesthetized with a mixture of Ketamine™ hydrochloride (MTC Pharmaceutical, Cambridge, Canada) and Xylazine™ (Havet Bayer Division, Etobicoke, Canada). After a midline laparotomy was performed, a colotomy was made in the distal transverse colon. Colonic mucosa was exposed and washed with mucomyst (Bristol Lab., Belleville, Canada) for two minutes to remove mucus, flushed with 1 percent methylene blue for three minutes, and scanned for the presence of ACF, by transillumination of the luminal surface at a magnification of 30 to 40X. In each rat, an isolated ACF containing four to eight crypts was identified and labeled. Labeling was accomplished by passing the 6-0 silk suture soaked in a green tattoo dye (Davidson Marking System™, Bradley Products Inc., Bloomington, MN) through the mucosa, 1 to 2 mm from the ACF both distally and proximally, leaving the ACF centered between the tattoo marks. Distance between the two tattoo marks did not exceed 3 mm, thus covering an area of no greater than 9 mm.² The soaked suture was passed far enough from the lesion to avoid any direct contact between the dye embedded in colonic wall and ACF. All TA were photographed. Colotomy was closed with continuous 6-0 proline sutures in one layer. Abdominal wall was closed with a continuous 4-0 suture and the skin with a 3-0 suture. After surgery, rats were maintained on 5 percent dextrose ad libitum for 48 hours. Their regular diet was reestablished on the third postoperative day.

One hundred days after the labeling procedure, a colonoscopy was performed under light Ketamine-Xylazine™ anesthesia, using a fiberoptic, pediatric bronchoscope. If a distal neoplasm was identified, the animal was killed by CO₂ asphyxiation, and the entire colon was examined for the presence of ACFs and macroscopic tumors. The TA was excised, fixed with formalin, and serially sectioned. Tumors identified in the TA were photographed, excised, and fixed in formalin.

If no visible lesion was identified in the distal colon during colonoscopy, animals were followed with subsequent endoscopies every four to six weeks up to 200 days after the tattooing procedure and then killed.

Group C (n = 30). Group C was a control group without carcinogen that received physiologic saline instead of AOM but was otherwise treated in the same manner as Group B.

Group D (n = 10). Group D was a second control group that was carcinogen-treated and tattooed in the same segment of the colon as animals in Group B, but the tattoo was in an area of normal mucosa that contained no evidence of ACF to ensure that the dye itself was not carcinogenic.

Not all animals survived the operative procedures. A total of 76 animals were initiated with AOM and were followed for tumors (Groups B and D), whereas 30 were given only saline (Group C). Survival through the procedures was 80 percent in Group A, 58 percent in Group B, 67 percent in Group C, and 90 percent in Group D. Animals that died did so during surgery from postoperative complications, predominantly colotomy-related early bowel obstructions, and were excluded from calculations. Most mortality occurred early in the study period and improved greatly as technical expertise was gained. The final number of animals completing the study was Group A (8), Group B (38), Group C (20), and Group D (9). Rats in Group B were the first to undergo the operative procedure, which accounted for the higher mortality in these animals.