A New Mouse Model for Evaluating the Immunotherapy of Human Colorectal Cancer

Heidi Hörig, Alberto Wainstein, Li Long, Doron Kahn, Sandeep Soni, Akiva Marcus, Winfried Edelmann, Raju Kucherlapati, and Howard L. Kaufman

ABSTRACT

A new murine model of human colorectal cancer was generated by crossing human carcinoembryonic antigen (CEA) transgenic mice (H-2Kb) with adenomatous polyposis coli (Apc1638N) knockout mice (H-2Kb). The resulting hybrid mice developed gastrointestinal polyps in 6–8 months that progressed to invasive carcinomas with a similar pattern of dysplasia and CEA expression as observed in human colorectal cancer. These animals exhibited incomplete or partial tolerance to CEA as evidenced by delayed growth of CEA-expressing tumors and the inability to inhibit CEA-specific CTL responses. These results have important implications for understanding the role of CEA-specific immunity in human colon cancer patients and suggest that vaccine strategies targeting CEA may be feasible. This model provides a powerful system for evaluating antigen-specific tumor immunity against spontaneous tumors arising in an orthotopic location and permits evaluation of therapeutic vaccine strategies for human colorectal cancer.

INTRODUCTION

Colorectal carcinoma remains a major public health threat, and new therapeutic strategies for prevention and treatment are needed. An improved understanding of tumor immunology has led to the development of several vaccine approaches targeted against specific human tumor antigens (1–4). The development of a therapeutic vaccine for colorectal cancer depends on the induction and maintenance of tumor-specific effector T cells capable of eliminating malignant cells. This process, as for priming any T-cell response, requires presentation of an antigenic epitope delivered by an immunogenic vector using an optimal schedule and route of administration. One of the major obstacles in vaccine development has been the lack of a relevant animal model for appropriately evaluating vaccine agents and immunization protocols. Furthermore, most animal models use transplantable tumors expressing xenointerrogens and do not replicate the spontaneous origin or localization of human colorectal tumors, thus providing little direct support for the design of clinical trials (5).

FAP3 is an autosomal dominant disorder characterized by the development of multiple colonic polyps and progression to colorectal carcinoma in virtually all of the patients without intervention (6). The recognition that FAP is attributable to the loss of the Apc gene guided the development of Apc knockout mice as a model of human colorectal cancer. Although the first such mice did develop multiple intestinal neoplasias, hence named Min, they survived <150 days and were difficult to use for prolonged therapeutic or immunological studies (7, 8). Whereas ApcMin mice contained a nonsense mutation at codon 850, most human Apc mutations occur within exon 15, the last exon. The Apc1638N knockout mouse was generated using a neomycin expression cassette inserted at codon 1638 resulting in an unstable truncated protein, although longer than the 850 amino acid protein in the ApcMin mouse (9). The Apc1638N mice develop aberrant crypt foci, polyps, and carcinomas of the small and large intestine, and live for up to 1 year, thus representing a more appropriate model of human gastrointestinal cancer and affording the opportunity to evaluate therapeutic interventions over a more realistic period of time (10).

CEA is one of the most frequently overexpressed oncofetal antigens and can be found in nearly all human colorectal tumors (11). CEA is a heavily glycosylated Mr 180,000 member of the immunoglobulin gene superfamily and may function as a homotypic adhesion molecule in the fetal colon (12). CEA is expressed at low levels in the normal adult colonic epithelium and other endodermally derived tissues but is highly overexpressed in neoplastic cells. In addition, tumor CEA differs from normal CEA expression in that it is aberrantly glycosylated, loses its typical apical localization, and is actively secreted by phospholipases resulting in high circulating serum levels (13). CEA has been suggested as a potential tumor antigen for vaccine development given its widespread expression pattern and accumulating evidence that CEA contains multiple HLA-restricted T-cell epitopes (14–16). Furthermore, recombinant poxviruses expressing CEA and CEA-pulsed dendritic cell vaccines have resulted in expansion of CEA-specific T-cell precursors and modest clinical responses in early-phase clinical trials (2, 17–19). Preclinical development of CEA vaccine strategies have relied on transplantable murine tumors transduced with human CEA ignoring the potential role of innate tolerance against CEA that is likely present in humans. This concern was partially addressed by the generation of a human CEA transgenic mouse model characterized by the expression of human CEA throughout the gastrointestinal tract (20). However, these mice do not develop polyps or gastrointestinal tumors, limiting the usefulness of this model for evaluating the immune response to CEA in the setting of de novo colon cancer growth.

To provide a more physiologically relevant model for understanding the role of CEA tolerance/immunity and CEA-directed immunotherapy in human colorectal cancer, we crossed the human CEA transgenic mice (H-2Kb) with the Apc1638N (H-2Kb) knockout mice. We report here that the hybrid Apc1638N/CEA transgenic mice spontaneously develop gastrointestinal CEA-expressing tumors, progressing from adenomatous polyps to invasive carcinomas over several months. We also demonstrate that these mice were able to inhibit growth of a CEA-bearing tumor, suggesting that tolerance did not occur. This is in contrast to the CEA transgenic mice that could not inhibit CEA-expressing tumors, suggesting that the development of tumors within the gastrointestinal tract may alter the host immune response to CEA. This model will be useful for additionally studying host-tumor interactions and the effects of gastrointestinal tumor growth on local mucosal and systemic antitumor immunity. The Apc1638N/CEA transgenic model will also be a powerful system for testing the efficacy of CEA-specific immunotherapy for human colorectal cancer.


MOUSE MODEL OF COLORECTAL CANCER

MATERIALS AND METHODS

Cell Lines. The murine colon adenocarcinoma cell lines MC38 and MC32a were generously provided by Jeffrey Schlom (NIH, Bethesda, MD) and maintained in DMEM containing 10% heat-inactivated FCS (Life Technologies, Inc., Rockville, MD). The MC32a cells were produced by transducing the MC38 cells with human CEA, as described elsewhere (21).

Generation of Apc1638N/CEA Hybrid Transgenic Mice. Apc1638N mice (H-2Kb) were generated using a neomycin expression cassette inserted at the position corresponding to codon 1638 resulting in a truncated Apc protein at amino acid 1638, as described previously (9). CEA transgenic mice (H-2Kb) were provided by Wolfgang Zimmermann (University of Freiberg, Freiburg, Germany), and have been described elsewhere (20). Heterozygous Apc1638N and CEA transgenic mice were crossed to produce the Apc1638N/CEA. Animals were maintained in filter top cages with ample access to food and water in the AALAC-accredited Animal Institute of the Albert Einstein College of Medicine.

Identification of Apc1638N and CEA Transgenes. Genomic DNA was extracted from the tail vein of newborn mice, digested, and analyzed for the transgenes by standard PCR. CEA was detected using primers designed to amplify the CEA1246–1352 gene segment and yielding a product of 106 bp, as described previously (20). The Apc1638N knock out was confirmed using Apc primers to the mutated Apc1638N allele and the wild-type Apc allele. The presence of the Apc1638N mutation was verified by finding two bands, 400 and 300 bp, as described elsewhere (9).

CEA Protein Detection. Mice were sacrificed, and organs removed and homogenized in tissue digestion buffer (1 ml of 400 mM NaCl/10 mM Tris Cl, pH 7.6/1 mM EDTA, pH 8.0, with phenylmethylsulfonyl fluoride (Sigma) at 20 μg/ml, aprotinin (Sigma) at 1 μg/ml, leupeptin (Sigma) at 10 μg/ml, and pepstatin (Sigma) at 10 μg/ml) (9). Protein concentration was determined by bicinchoninic acid protein assay (Pierce, Rockford, IL), and normalized protein was analyzed by standard immunoblot using the rabbit antihuman CEA polyclonal antibody (DAKO, Carpinteria, CA). The human colon carcinoma cell line GEO (from Judith Kantor, NIH, Bethesda, MD) was used as a positive control. CEA from fecal pellets was extracted in PBS containing 1 Triton X-100 (Bio-Rad Laboratories, Hercules, CA) and protein content determined by bicinchoninic acid (Pierce). CEA was identified by standard ELISA using the mouse antihuman CEA mAb, COL-1 (Zymed, South San Francisco, CA) for coating (1:1000). CEA in fecal extracts (1:50) was detected with a rabbit antihuman CEA polyclonal antibody (1:10000), a secondary horse radish peroxidase-conjugated antirabbit antibody (Jackson Immune Research Laboratories, West Grove, PA) and color developed with Diaminobenzidine. CEA (dilutions 1:200 to 1:6400) was used as a positive control. After incubation, plates were washed several times with Tris containing 0.1% Tween 20. Cytotoxic assay. Cytotoxicity was determined in a 6-h chromium (51Cr) release assay using splenocytes from C57BL/6 and Apc1638N/CEA mice bearing MC32a tumors. Effector cells were prepared from whole spleens by macerating using frosted glass slides, and red cells were lysed with ACK lysis buffer. Target cells consisted of the CEA-expressing MC32a or the CEA-negative MC38 cells (3 × 106 – 6 × 106), labeled with 200 μCi 51Cr (DuPont, Boston, MA) for 1 h at 37°C. Labeled targets were washed and resuspended at 3 × 106 cells/ml and plated in 100-μl aliquots mixed with an equal volume of effector cells (added in serial dilution of E:T from 100:1 to 125:1) in triplicate using 96-well U-bottomed plates. After incubation, plates were centrifuged, and 25 μl of supernatants were mixed with 150 μl of SuperMix (Perkin-Elmer, Boston, MA) and counted using a MicroBeta TriLux liquid scintillation counter (Wallac-Perkin-Elmer, Boston, MA). The percentage of specific 51Cr release was calculated as: 100 × (experimental release – spontaneous release)/maximum release – spontaneous release. Spontaneous release was determined using target cells without effectors, and maximum release was determined by adding 1% Triton X-100 to target cells. The assay was repeated twice with similar results.

RESULTS

Generation and Characterization of Apc1638N/CEA Mice. The Apc1638N mouse (H-2Kb) was generated by insertion knockout technology and results in expression of a truncated Apc protein product of 1638 amino acids, as described elsewhere (9). The CEA transgenic mice were generated by microinjection of a cosmid clone encompassing the complete human CEA gene into the male pronucleus of fertilized mouse oocytes derived from C57BL/6 (H-2Kb) mice, as reported previously (20). We crossed these mice and confirmed hybrid Apc1638N/CEA mice by transgene PCR analysis of tail DNA. The Apc1638N/CEA mice before and after sacrifice and weighed. All of the experiments were repeated three times, and one representative result is shown.

In Vivo Tumor Studies. Mice (8 weeks of age) were implanted s.c. with 2 × 106 MC32a (CEA+) or MC38 (CEA-) cells. MC32a cells were routinely sorted for >80% CEA expression using the COL-1 mAb (Zymed) and a secondary FITC-labeled goat-antimouse IgG antibody (Sigma Chemical Co.) by fluorescence-activated cell sorting using a FACSVantage SE instrument (Becton Dickinson, San Jose, CA). Tumor growth was monitored daily, and tumor volumes were calculated as follows: area (mm2) = (short axis)2 × (long axis)/2. Tumors were excised after sacrifice and weighed. All of the experiments were repeated three times, and one representative result is shown.

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vein DNA from newborn pups (data not shown). CEA protein expression was determined by Western blot analysis of tissue lysates and was found in the stomach, small intestine, and colon of the Apc1638N/CEA mice (Fig. 1). CEA expression was not observed in any other organ including the lungs, liver, spleen, and kidneys. The highest level of expression of CEA was seen in the colon and correlates with typical CEA expression patterns in humans. These data indicate that Apc1638N/CEA mice express human CEA in a tissue-specific manner, as expected by the presence of the human regulatory elements for correct spatiotemporal expression in the genomic DNA fragment used to generate the CEA transgenic mice and the recognition of these elements by murine trans-acting factors (22).

To additionally characterize the pattern of CEA expression in the Apc1638N/CEA mice, fecal and serum samples were collected and CEA protein content determined by ELISA assay using the mouse anti-human CEA mAb 32E1. CEA was present in the feces of mice, and the concentration varied between individual animals, as reported previously in the CEA transgenic mice (20). In contrast, serum CEA was not detected until Apc1638N/CEA mice were 4 months of age and then increased as the mice aged (Table 1). This pattern of increasing serum CEA correlates with progression of polyp growth (see below) and mimics the human situation, wherein low levels of CEA are found in normal individuals and increase with tumor burden and stage of disease in colorectal cancer patients (23). We also determined the presence of fecal occult blood in the Apc1638N/CEA mice and their age-matched nontransgenic littermates. Interestingly, all of the Apc1638N/CEA mice had occult blood on fecal examination as early as 2 months providing a simple and sensitive screening method for identifying transgenic mice and early polyp formation (data not shown). The presence of occult blood has also been reported in patients harboring adenomatous colorectal polyps (24). Thus, the hybrid Apc1638N/CEA mice express CEA in the gastrointestinal tract, shed CEA and microscopic blood into the gut lumen, and demonstrate elevated serum levels that correlate with increasing age and tumor growth, consistent with colorectal cancer progression in humans.

Gastrointestinal Neoplasia in the Apc1638N/CEA Mice. To examine the effect of the Apc1638N gene on intestinal polyp and tumor formation in Apc1638N/CEA mice, we randomly analyzed the gastrointestinal tract of mice every 2 months. The Apc1638N/CEA mice demonstrated an age-dependent increase in polyp formation, as manifested by an increase in the number, size, and histological severity of the polyps (Fig. 2). A careful histological examination of the gastrointestinal tract failed to detect any polyps in 2-month-old mice, whereas by 4 months we observed 1–7 polyps and by 6 months all of the mice had between 4–13 polyps (Fig. 2a). Although the number of polyps did not increase substantially between 6 and 8 months, the polyps did increase in size (Fig. 2b). The polyps were scattered throughout the gastrointestinal tract, occurring predominantly in the small intestine and, less frequently, in the stomach and colon (Table 2). Histopathological examination of the polyps in the 4-month-old mice revealed areas of polypoid hyperplasia with small spherical elevations of the mucosa to a height twice that of the adjacent flat mucosa. Progression to adenoma with severe dysplasia was seen in 6-month-old Apc1638N/CEA mice, similar to a typical human colorectal adenoma (Fig. 3a). By 8 months of age, the Apc1638N/CEA mice displayed multiple areas of polypoid hyperplasia and adenomas but now also developed adenocarcinomas, characterized by invasion of the muscularis propria (Fig. 3b). The distribution of hyperplastic polyps, adenomas, and invasive carcinomas for 10 different 8-month-old Apc1638N/CEA mice is shown in Table 2. A heterogeneous pattern was seen with multiple synchronous tumors scattered throughout the gastrointestinal tract. Generally only one carcinoma was found, although one mouse (Table 2, Animal 9) did develop two separate invasive cancers. The diverse pattern of tumors found in the gut is reminiscent of human FAP and even sporadic colorectal cancer, where multiple polyps are common, and carcinomas can arise throughout the length of the large intestine. Whereas some mice were able to survive up to 12 months, older Apc1638N/CEA mice developed a characteristic chronic illness manifested by weight loss, shaggy coat appearance, and lethargy.

![Figure 2](https://example.com/fig2.png)

**Fig. 2.** The number (a) and size (b) of macroscopically visible polyps in the gastrointestinal tract of Apc1638N/CEA mice increase with age. Random mice were sacrificed every 2 months, and the number of polyps identified by a blinded observer were recorded. The average for the number and area of polyps for all mice evaluated is shown; bars, ±SD.

<table>
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<tr>
<th>Table 2</th>
<th>Nature and incidence of tumors in 8-month-old Apc1638N/CEA mice</th>
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<td>Animal</td>
<td>Stomach</td>
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*P, hyperplastic polyp; A, benign adenoma; CA, invasive carcinoma.

![Table 1](https://example.com/tab1.png)

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<tr>
<th>Table 1</th>
<th>CEA protein in serum and feces of Apc1638N/CEA mice</th>
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<tr>
<td>Mouse no.</td>
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*ND, not determined.
CEA expression on the tumor tissue was detected by immunohistochemistry using an anti-CEA mAb. CEA was highly expressed by the malignant tissue of the Apc\(^{1638N}/\)CEA mice (Fig. 3c), as compared with low-level expression on nonmalignant colon tissue from the same mice (data not shown). Thus, the Apc\(^{1638N}/\)CEA mice develop spontaneous gastrointestinal tract polyps around 4 months of age, which increase in number for another 2 months. The tumors begin as hyperplastic polyps and progress through increasingly dysplastic adenomas, and ultimately become invasive carcinomas by 8 months of age. The tumors also express human CEA, which appears to be highly expressed in the more severely dysplastic and invasive tumors. Thus, this model represents the typical features of human FAP wherein multiple polyps occur and progress in an orderly fashion to frank CEA-expressing carcinomas over time.

**Apc\(^{1638N}/\)CEA Mice Are Incompletely Tolerant to CEA.** The Apc\(^{1638N}/\)CEA model provides an opportunity to evaluate how the immune system responds to overexpression of the self-antigen CEA by a spontaneous tumor arising in the gastrointestinal tract. To ascertain the degree of tolerance to CEA in this model, we evaluated humoral and cellular CEA-specific immunity in Apc\(^{1638N}/\)CEA with and without a tumor burden. In accord with previous studies in CEA transgenic mice, we were unable to detect the presence of anti-CEA antibodies in the Apc\(^{1638N}/\)CEA mice, even after challenge with a CEA-expressing murine colon carcinoma (data not shown). Therefore, the mice are tolerant with respect to anti-CEA antibody production.

We next sought to determine whether the Apc\(^{1638N}/\)CEA mice would allow the growth of a transplantable CEA-expressing tumor. Previous studies have demonstrated that growth of the MC32a murine colon carcinoma cell line expressing human CEA is significantly inhibited in normal C57BL/6 mice because of induction of CEA-specific T-cell responses (25). Furthermore, CEA transgenic mice are tolerant to challenge with the MC32a cell line and do not exhibit CEA-specific CTL (26). Surprisingly, growth of the MC32a tumor was delayed in both the Apc\(^{1638N}/\)CEA and C57BL/6 mice, suggesting that, unlike CEA transgenic mice, the Apc\(^{1638N}/\)CEA transgenic mice are not completely tolerant to human CEA (Fig. 4a). Because the growth inhibition of the MC32a tumor in C57BL/6 mice has been associated with CEA-specific CTL responses, we sought to determine whether CTL responses were also present in the Apc\(^{1638N}/\)CEA mice. There was no difference in CEA-specific CTL responses in the Apc\(^{1638N}/\)CEA mice compared with normal C57BL/6 mice (Fig. 4b). These results suggest that the mice are not tolerant with respect to T-cell immunity, are able to recognize CEA, and inhibit tumor growth in a CEA-specific manner.

To more completely compare these findings to the CEA transgenic mice, we evaluated the growth of the MC32a tumor line in the CEA transgenic, Apc\(^{1638N}/\)CEA, Apc\(^{1638N}/\)CEA, and normal C57BL/6 mice. Although tumors in the Apc\(^{1638N}/\)CEA mice grew slightly faster than those in normal or Apc\(^{1638N}/\)CEA mice, they were significantly delayed compared with the CEA transgenic mice (Fig. 5). These results suggest that the Apc\(^{1638N}/\)CEA mice exhibit humoral but not T-cell tolerance to CEA.

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Fig. 3. Neoplastic lesions and pattern of CEA expression in Apc\(^{1638N}/\)CEA mice. a, H&E staining showing a typical adenoma; b, an adenocarcinoma in the small intestine of an 8-month-old Apc\(^{1638N}/\)CEA mouse. Note the area of invasion through the lamina propria (arrow). c, immunohistochemical staining with an anti-CEA mAb of a small intestinal polyp in an 8-month-old Apc\(^{1638N}/\)CEA mouse. Note the heavier staining of the adenoma compared with the normal gut epithelium.
shown is one result from two separate experiments; CEA-negative MC38 cells as targets. There was no difference between the two groups. Tumors were evaluated in a 6-h chromium release assay using MC32a (squares) and Apc1638N/CEA (open circles) bearing the CEA-expressing MC32a carcinoma. Apc1638N/CEA mice develop spontaneous gastrointestinal tumors within 40 days of birth, and the animals died within 100–130 days because of pyloric obstruction. Additionally, these mice developed lymphomas and sarcomas additionally limiting long-term survival studies. In contrast, our mice lived for up to 12 months, providing a CEA-expressing spontaneous gastrointestinal tumor model for evaluating CEA-specific immunity and immunotherapy.

Tumor immunity depends on the generation of tumor-specific T-cell responses. Recently, Zinkernagel (33) has postulated that priming of T-cell responses depends on the dose and structure of antigen, as well as the location and timing of antigen presentation. This paradigm of T-cell activation provides a framework for evaluating the induction of CEA-specific T-cell responses under physiological conditions and, secondly, for optimizing vaccine strategies. Although generally considered weakly immunogenic, CEA is overexpressed in >95% of human colorectal cancers (34). CEA is present at low levels and localized to the luminal surface of normal adult colonicocytes but is found in a disordered pattern throughout the cell membrane of malignant cells where it may disrupt intercellular adhesion resulting in disorganized growth and movement of transformed cells (35). Because CEA is not expressed in the thymus of transgenic mice, there is no a priori reason to expect that CEA-reactive cells will be deleted by negative thymic selection (20, 36). Thus, the lack of CEA-reactive T cells under normal physiological conditions is likely attributable to tumors, they do not express CEA or other known tumor-associated antigens, thus limiting their usefulness. An attempt to cross the CEA transgenic mice with ApcMin mice did result in spontaneous CEA-expressing tumors, but the mice had a life span of <60 days making tumor treatment studies impossible (31). A similar problem was encountered in a model using a CEA promoter/SV40 T-antigen gene construct resulting in CEA-expressing gastric carcinomas (32). These mice developed gastric tumors within 40 days of birth, and the animals died within 100–130 days because of pyloric obstruction. Additionally, these mice developed lymphomas and sarcomas additionally limiting long-term survival studies. In contrast, our mice lived for up to 12 months, providing a CEA-expressing spontaneous gastrointestinal tumor model for evaluating CEA-specific immunity and immunotherapy.

Discussion

Improvements in our understanding of basic immunology and the lack of effective therapy have focused attention on the development of vaccines for colorectal cancer. An essential component in the development of any therapeutic strategy is the demonstration of efficacy in an animal model. Various vaccine approaches targeting CEA have been proposed with evidence for clinical effectiveness provided by murine studies using transplantable tumors expressing human CEA, making appropriate immunological analyses difficult (26–28). To provide a more relevant animal model, we crossed the tumor-prone Apc1638N mice (H-2Kb) with human CEA transgenic mice (also H-2Kb). Hybrid Apc1638N/CEA mice develop spontaneous gastrointestinal tumors that increase in number and size with age, and progress from hyperplastic polyps to invasive carcinomas between 4 and 8 months of age, in a manner similar to that reported in human polyposis syndromes and colorectal cancer. In addition, these mice express human CEA as a self-antigen exclusively in the gastrointestinal tract in a pattern that resembles human CEA expression. In fact, we observed higher CEA staining on malignant tissue and found elevated serum CEA in older mice harboring established tumors. Whereas the distribution of the polyps was largely confined to the small intestine, this is not unexpected, because FAP patients often exhibit extraluminal polyps, particularly in the upper gastrointestinal tract depending on the location of the specific Apc mutation (29).

Other models have been proposed for evaluating CEA immunity and human colorectal cancer. The CEA transgenic mice have been used to evaluate the ability of various vaccine strategies to break tolerance, although these mice do not develop spontaneous tumors (26, 30). Furthermore, these mice cannot be used to evaluate the effect of therapeutic interventions on tumors within the gastrointestinal tract. Whereas the ApcMin and Apc1638N mice develop gastrointestinal...
peripheral tolerance, although little is known about how such tolerance is maintained. We actually observed significant inhibition of CEA-bearing tumors in the ApcMin/N(CEA) mice and did not see a reduction in CEA-specific CTL responses compared with normal C57BL/6 mice. This finding is in agreement with the identification of HLA-restricted T-cell epitopes within CEA and the demonstration of CEA-specific T cells derived from normal human donors using in vitro CEA exposure or from colorectal cancer patients immunized with CEA-targeted vaccines (1, 2, 17–19, 37). The recent report that apopotic colonic epithelial cells may be sampled by gut mucosal dendritic cells and processed in mesenteric lymphoid tissue raises the possibility that CEA may be presented, for either immunizing or tolerizing purposes, through this pathway (38). Whereas it is possible that early polyp formation may alter T-cell tolerance induction, it is also plausible that the presence of the ApcMin/N gene may influence the immune response to CEA. The maintenance of self-tolerance mediated by T-cell unresponsiveness may also be secondary to clonal deletion, anergy, ignorance, shifts in local cytokine production, changes in the dose of antigen, or other factors (36, 39). The ApcMin/N(CEA) model provides an excellent system for additionally elucidating the mechanism of peripheral CEA tolerance during colorectal cancer progression, and we are actively pursuing these studies.

The ApcMin/N(CEA) mice also provide an excellent model for evaluating CEA-directed vaccine strategies. Although most vaccine approaches have focused on the induction of systemic CEA-specific T cells, little information exists about the activity of such cells at the site of tumor growth. This may be especially important when the tumor is located in the gastrointestinal tract, because it has been shown that mucosal sites may remain naïve after systemic vaccinia virus exposure (40). An effective vaccine for colorectal cancer requires optimizing the antigen, vector, timing, and route of administration to achieve sufficient T-cell activation for tumor rejection. The ApcMin/N(CEA) mice permit the evaluation of CEA-directed vaccines in a model where tumor growth and CEA expression are colocalized. Whereas the model is more representative of human FAP rather than sporadic colorectal cancer, somatic Apc mutations and deletions are involved in the initiation of nearly all human colorectal carcinomas (29). Thus, the murine model described in this report should prove useful in better understanding the normal host response to CEA and guiding the continued development of vaccine strategies for human colorectal cancer.

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REFERENCES


A New Mouse Model for Evaluating the Immunotherapy of Human Colorectal Cancer

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