[CANCER RESEARCH 54, 4169–4176, August 1, 1994]

Mice Transgenic for the Human Carcinoembryonic Antigen Gene Maintain Its Spatiotemporal Expression Pattern[1,2]

Anne-Marie Eades-Perner,[3] Herman van der Putten, Antje Hirth, John Thompson, Michael Neumaier, Sabine von Kleist, and Wolfgang Zimmermann

Institute of Immunobiology, University of Freiburg, D-79104 Freiburg, Germany [A-M. E-P., A. H., J. T., S. v. K., W. Z.]; Ciba-Geigy, CH-4002 Basel, Switzerland [H. v. d. P.]; and University Hospital Eppendorf, D-20251 Hamburg, Germany [M. N.]

ABSTRACT

The tumor marker carcinoembryonic antigen (CEA) is predominantly expressed in epithelial cells along the gastrointestinal tract and in a variety of adenocarcinomas. As a basis for investigating its *in vivo* regulation and for establishing an animal model for tumor immunotherapy, transgenic mice were generated with a 33-kilobase cosmid clone insert containing the complete human *CEA* gene and flanking sequences. CEA was found in the tongue, esophagus, stomach, small intestine, cecum, colon, and trachea and at low levels in the lung, testis, and uterus of adult mice of independent transgenic strains. CEA was first detected at day 10.5 of embryonic development (embryonic day 10.5) in primary trophoblast giant cells and was found in the developing gut, urethra, trachea, lung, and nucleus pulposus of the vertebral column from embryonic day 14.5 onwards. From embryonic day 16.5 CEA was also visible in the nasal mucosa and tongue. Because this spatiotemporal expression pattern correlates well with that known for humans, it follows that the transferred genomic region contains all of the regulatory elements required for the correct expression of *CEA*. Furthermore, although mice apparently lack an endogenous *CEA* gene, the entire repertoire of transcription factors necessary for correct expression of the *CEA* transgene is conserved between mice and humans. After tumor induction, these immunocompetent mice will serve as a model for optimizing various forms of immunotherapy, using CEA as a target antigen.

INTRODUCTION

CEA[4] is a well known tumor marker for a variety of adenocarcinomas, especially colorectal tumors. Despite its presence in certain normal tissues, increased serum concentrations have made it a useful indicater of residual disease or tumor recurrences in the postoperative surveillance of tumor patients following resection (1). Because CEA is located in the plasma membrane of tumor cells, it can also be used for targeting CEA-specific mAb in the radio-immunolocalization or immunotherapy of CEA-expressing tumors and their metastases (2, 3). However, a number of problems exist that still limit the usage of CEA for tumor targeting. These include antibody penetration of tumors, liver and kidney uptake of antibodies, and antibody binding to normal tissues that express CEA, which could lead to autoimmune responses or tissue destruction through cytotoxic antibody conjugates (2).

Molecular cloning has revealed that *CEA* belongs to a gene family consisting of at least 22 genes (4) which are part of the immunoglobulin gene superfamily. Sequence comparisons identify two main subgroups. The CEA subgroup members are mainly membrane bound, either as integral membrane proteins, *e.g.*, BGP, or after post-translational modification, via a glycosyl phosphatidylinositol moiety, as found for CEA (5). *In vitro*, CEA subgroup members convey homo- and heterophilic cell adhesion (6, 7). The second subgroup consists of 11 genes encoding the secreted pregnancy-specific glycoproteins of unknown function. *CEA* gene families have also been characterized in rodents (8–10). Despite homology, it has proven difficult to determine rodent counterparts for individual human genes through sequence comparisons. Surprisingly, no *CEA* homologue has thus far been identified; in fact, no rodent CEA-related molecules containing a glycosyl phosphatidylinositol anchor have been characterized to date.

CEA family members in normal tissues show distinct expression patterns (5). Immunohistochemistry has revealed that CEA is mainly restricted to distinct regions of the gastrointestinal tract (11). Various other members of the CEA subgroup, *e.g.*, nonspecific cross-reacting antigen and BGP, are often coexpressed with CEA in the colonic mucosa. However, nonspecific cross-reacting antigen and BGP are also found in maturing granulocytes that do not express CEA (5, 12). In addition, BGP is expressed in epithelia of bile canaliculi (5). Pregnancy-specific glycoprotein subgroup members are synthesized mainly in the syncytiotrophoblast cells of the placenta (13).

In an attempt to determine the elements responsible for regulating the expression of *CEA* at the transcriptional level, we previously described the isolation of a cosmid clone containing the complete coding region of the *CEA* gene, including 3.3 kilobases of the 5′-flanking region and 5 kilobases of the 3′-flanking region (14). After stable transfection of Chinese hamster ovary cells with this cosmid clone, membrane-bound CEA was expressed under the control of its own *cis*-regulatory elements, which were recognized by the rodent *trans*-acting factors (15). Functional analyses in a transient transfection assay allowed identification of a 424-base pair region, upstream from the translational start site, that apparently allows cell type-specific expression of the *CEA* gene (14).

In this communication, we have used the complete insert of the same CEA cosmid clone to generate transgenic mice that stably express human CEA. These studies present a basis for identification of the elements responsible for regulating the spatiotemporal expression pattern of *CEA in vivo*, both during fetal development and in adult mice. Additionally, the *CEA* promoter may be useful in directing the expression of different oncogenes to epithelial cells so that their roles in tumorigenesis of organs belonging to the gastrointestinal tract may be better understood. Finally, after induction of tumors in *CEA*-transgenic mice, the usefulness and possible unwanted side effects of CEA as a target antigen for immunotherapy of CEA-producing tumors can be investigated in these immunocompetent mice.

MATERIALS AND METHODS

Generation of Transgenic Mice. The cosmid clone cosCEA1, encompassing the complete human *CEA* gene, has been described previously (14). A 33-kilobase *Aat*II DNA fragment from cosCEA1 containing the *CEA* gene as well as 0.6 kilobase and 0.1 kilobase of vector sequences at the 5′- and 3′-ends, respectively, was isolated using the freeze-squeeze method (16). A solution containing 2.5 μg/ml levels of this fragment in 5 mM Tris, 0.1 mM EDTA, pH 7.4, was used for microinjection into the male pronucleus of fertilized mouse

Received 2/10/94; accepted 5/24/94.

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 grants from the Deutsche Forschungsgemeinschaft and the Dr. Mildred Scheel Stiftung für Krebsforschung.

[2] This paper is dedicated to Peter Sitte on the occasion of his 65th birthday.

[3] To whom requests for reprints should be addressed, at Institut für Immunbiologie der Universität, Stefan-Meier-Strasse 8, D-79104 Freiburg, Germany.

[4] The abbreviations used are: CEA, carcinoembryonic antigen; BGP, biliary glycoprotein; PBS, phosphate-buffered saline; mAb, monoclonal antibody; cDNA, complementary DNA; ELISA, enzyme-linked immunosorbent assay; EIA, enzyme immunoassay.

oocytes derived from C57BL/6 × CB6 F_1 mice (Ciba Animal Breeding Center, Basel, Switzerland), as described (17). Lines were established from founder animals by mating with C57BL/6 mice. Transgenic mice were identified either by Southern blot analysis of tail DNA or by analysis of fecal extract (see below).

Southern Blot Analyses. Tail DNA was isolated as described previously (17). Standard Southern blot analysis was performed (18), using *Eco*RI-digested ^{32}P-labeled cosCEA1 as a probe. The probe was random-primed with the Megaprime kit (Amersham-Buchler, Braunschweig, Germany). Final washings were carried out at 66°C in 0.1× SSPE, 0.1% sodium dodecyl sulfate (1× SSPE is 0.18 M NaCl, 10 mM sodium phosphate, pH 7.4, 1 mM EDTA). Under these conditions no cross-hybridization occurred beween the Alu elements present in the probe and mouse repetitive sequences. For determination of transgene copy number, 10 μg of mouse tail DNA were digested with *Sac*I and hybridized in a standard Southern blot with a ^{32}P-labeled 4.8-kilobase *Sac*I DNA fragment of cosCEA1 (see Fig. 1, *bottom*) and a ^{32}P-labeled 2.2-kilobase *Eco*RI-*Sac*I DNA fragment from a genomic clone (pMT1) containing the mouse *Thy-1* gene, and the filters were washed under stringent conditions as described above. The hybridization signals were quantified using a Bio-Imaging BAS1000 analyzer (Fuji, Tokyo, Japan). The number of *CEA* gene copies was calculated by comparing the signal strength obtained with the *CEA* probe with that of the single-copy *Thy-1* gene (19).

RNA Isolation and Detection. Total cellular RNA was isolated from organs (either fresh or frozen at −70°C) according to the method of Chomczynski and Sacchi (20). Standard Northern blot hybridization procedures were performed (18). As a probe, the CEA cDNA insert lacking the 3′-untranslated region (21) was excised with *Hind*III from plasmid pBEHCEA (22) and radiolabeled as described above. Hybridizations were also carried out using a ^{32}P-labeled mouse β-actin cDNA (23) to ascertain the intactness of the RNA. Filters were washed under stringent conditions as described above.

Immunohistochemical Analyses. Organs isolated from adult mice (2–12 months of age), which had been anesthetized with Forene (Abbott GmbH, Wiesbaden, Germany) and killed by cervical dislocation, either were frozen immediately in isopentane at −70°C and embedded in Jung freeze medium (Leica Instruments, Nuβloch, Germany) diluted with 2 volumes of water or were first fixed in 4% (w/v) paraformaldehyde in PBS at 4°C for 24–48 h and then incubated for 24 h in 0.5 M sucrose in PBS at 4°C. Cryostat sections (6–7-μm thick) of tissues were fixed in acetone for 10 min at room temperature. Endogenous peroxidase activity was inhibited by first incubating sections in methanol, 0.3% hydrogen peroxide, for 30 min. The CEA-specific chimeric mAb cT84.66 (24) was used either in complete form or as a Fab fragment. Binding of the primary antibody was followed by incubation with horseradish peroxidase-conjugated rabbit anti-human IgG (Dianova, Hamburg, Germany). The staining reaction was performed using the substrate 3,3′-diaminobenzidine tetrahydrochloride. Sections were counterstained with hematoxylin. Staged embryos and placentae were treated likewise. Earlier stage embryos (embryonic days 8.5–12.5) were not dissected from the uterine tissue but were sectioned *in toto*. Staged embryos (embryonic days 8.5–19.5) were obtained from superovulated nontransgenic females fertilized by transgenic males. Midday after vaginal plug formation was designated embryonic day 0.5.

Tissue Protein Extracts. Tissue from non-neoplastic regions of resected colon from tumor patients (University Hospital, Freiburg, Germany) and mouse colon tissue were pulverized under liquid N_2 and resuspended in PBS, 0.5% Nonidet P-40, 2 mM phenylmethylsulfonyl fluoride (1 ml/g frozen tissue). The cells were lysed by ultrasonification using a Branson B-12 sonifier (Heinemann, Schwäbisch-Gmünd, Germany) with 5 pulses of 15 s each at 40 W. The homogenate was centrifuged at 13,000 × *g* for 30 min at 4°C and the clear supernatant was stored at −20°C. Protein concentration was determined using the Bio-Rad (Munich, Germany) assay and bovine serum albumin standards.

CEA Quantitation. Fecal pellets stored at −20°C were extracted by resuspending the pellets in 2 volumes of PBS, 1% Triton X-100, incubating the mixture for 5 min at room temperature, and saving the supernatant following centrifugation at 13,000 × *g* for 10 min at room temperature. The pellet was reextracted with an equal volume of PBS, 1% Triton X-100, and the supernatants were combined. Levels of CEA in extracts from mouse feces were determined with a CEA EIA kit (Hoffmann LaRoche, Basle, Switzerland), following the manufacturer's instructions. Determination of the CEA content in tissue extracts, human feces, and mouse serum was performed with a CEA-specific sandwich ELISA using combinations of the following mAbs: Tet2, D14HD11, 26/3/13, and 26/5/1 (22).

RESULTS

Establishment of Mice Transgenic for the Human *CEA* Gene. The *CEA* transcription unit, flanked by 3.3 kilobases of 5′-sequence and 5 kilobases of 3′-sequence, is contained within cosCEA1 (Fig. 1) (14). An *Aat*II DNA fragment containing the insert from cosCEA1 was used to generate transgenic mice. Of the 36 mice born, 12 were found to contain the human *CEA* gene. The Southern blot analysis of nine animals is shown in Fig. 1. Eight of these mice have intact copies of the cosmid insert, since all expected fragments could be detected (Fig. 1, *top*, compare *lanes 1–8* with *lane 10*). The presence of a 3.9-kilobase *Sst*I DNA fragment in the different transgenic lines indicates a preferential head-to-tail arrangement of the multiple insert copies in tandem. The lack of this fragment and the presence of only two end fragments in animal 2676 indicate the existence of only a single-copy transgene (Fig. 1, *top*, *lane 2*). No hybridization signals were observed with DNA of a nontransgenic littermate (Fig. 1, *top*, *lane 9*). Of the 12 mice which were found to be transgenic for human *CEA*, eight transmitted this gene to their offspring. Four of these mice

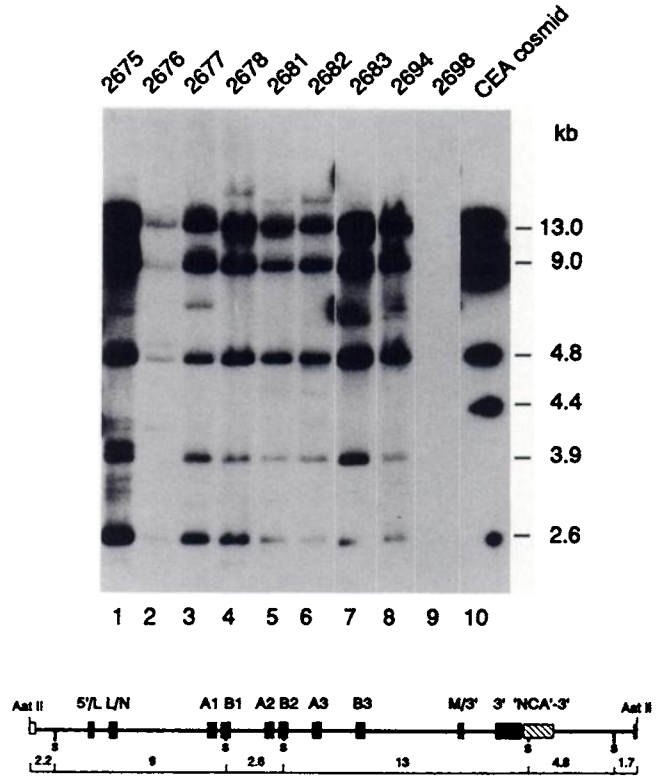

Fig. 1. Identification of transgenic mice by Southern blot analysis. *Top*, tail DNA of founder animals (numbered at the top of each lane) was digested with *Sst*I and subjected to standard Southern blot analysis using ^{32}P-labeled cosCEA1 DNA as the probe. Fragment sizes are indicated to the right of the blot. cosCEA1 DNA was also digested with *Sst*I and included as a positive control (*lane 10*). Note that vector sequences from cosCEA1 are contained within the 4.4-kilobase fragment and one of the 9.0-kilobase fragments. The 3.9-kilobase fragment in *lanes 1* and *3–8*, which is the summation of the 2.2-kilobase and 1.7-kilobase *Aat*II/*Sst*I fragments (see *bottom*), reflects a head-to-tail orientation of multiple transgene copies in tandem. Note more weakly hybridizing DNA fragments, which probably contain cosmid end fragments and flanking mouse genomic DNA sequences. *Bottom*, the *Sst*I restriction enzyme map of the *Aat*II DNA fragment of cosCEA1 is shown. *Numbers*, size of the fragments (in kilobases). *Black boxes*, exons. *Striped box*, potential 3′-exon. *5′*, 5′-noncoding region; *L*, leader; *N*, N (IgV-like)-domain; *A1–3* and *B1–3*, IgC-like domain of subtypes A and B; *M*, membrane domain; *3′*, 3′-noncoding region. *Open boxes*, vector sequences. '*NCA*', nonspecific cross-reacting antigen-like; *S*, *Sst*I.

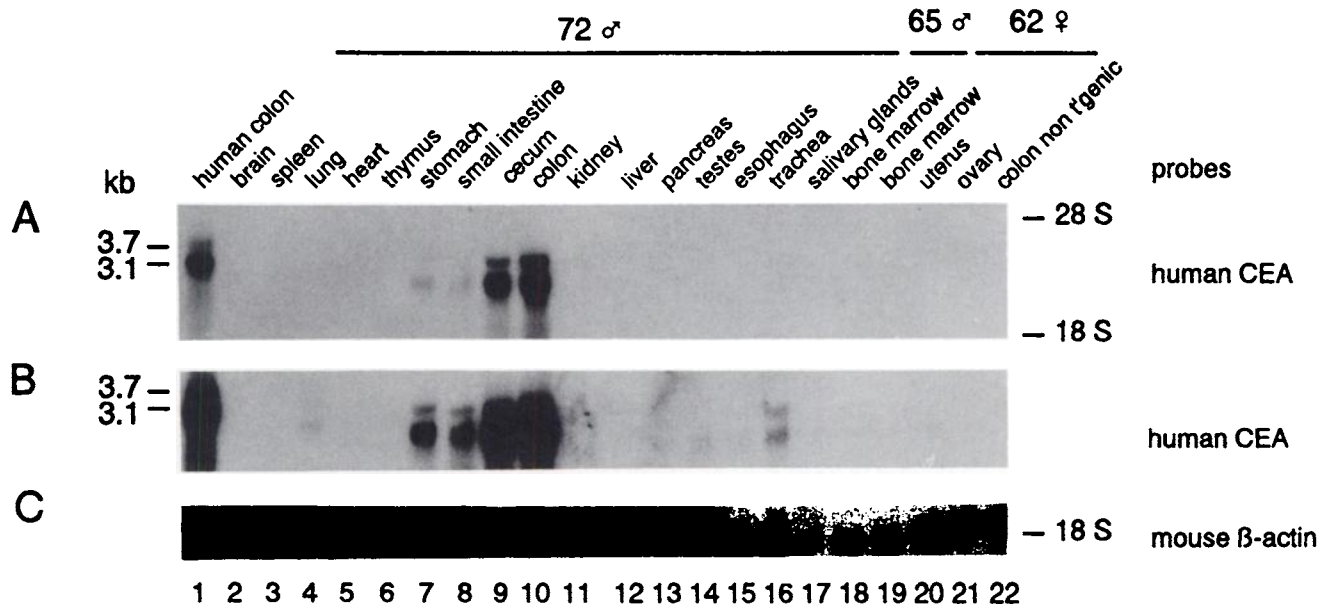

Fig. 2. Expression of human CEA mRNA in transgenic mice. *A*, 10 μg of total RNA from a human and a nontransgenic mouse colon, along with various organs from three different transgenic mice (mice 72, 65, and 62 from line 2683), were electrophoresed in a formaldehyde-agarose gel and transferred to a nylon membrane. The RNAs were hybridized with a ^{32}P-labeled probe covering the full-length CEA cDNA. The sizes of the CEA transcripts are indicated to the left of the blot. In the right margin, the positions of the 28S and 18S rRNAs are shown. *B*, longer exposure of *A*. *C*, after decay of the CEA probe, the blot was rehybridized with a mouse β-actin probe.

(mice 2681, 2682, 2683, and 2694) were used to establish independent lines transgenic for *CEA* and were analyzed in detail.

Analysis of *CEA* Expression at the mRNA and Protein Levels. Northern blot analysis of RNA isolated from different organs of transgenic mice revealed that *CEA* was not ubiquitously expressed (Fig. 2). There are two CEA mRNA species in normal human colon (Fig. 2*A*, *lane 1*), a 3.1-kilobase transcript and a less abundant 3.7-kilobase transcript, due to alternate polyadenylation (25). Both products were also found in the transgenic mice, indicating that the transgene is being transcribed correctly. The expression pattern was found to be essentially identical for the four independently established lines transgenic for *CEA*. High levels of CEA mRNA were found in colon and cecum (Fig. 2*A*, *lanes 9* and *10*), whereas lower levels were present in stomach and small intestine (Fig. 2*A*, *lanes 7* and *8*). In addition, very low levels were found in the lung, trachea, testis, and esophagus (Fig. 2*B*, *lanes 4* and *14–16*) but not in any other organ tested (Fig. 2, *A* and *B*; see Table 2). Although not visible in Fig. 2, after an extended exposure marginal hybridization signals were also observed with uterus RNA. No hybridization signal was obtained with RNA from the colon of a nontransgenic mouse (Fig. 2*A*, *lane 22*).

The steady state levels of CEA mRNA in the colons of the four different lines were compared with each other and with those of two human colons in Northern blot analyses (Fig. 3). Although equal amounts of RNA were loaded, variability in the levels between transgenic lines was obvious. Compared with human colon, the mRNA levels were lower in lines 2681 (mouse 3011) and 2683 (mouse 3061) and approximately equal or slightly higher in lines 2682 (mouse 3004) and 2694 (mouse 3077). These expression levels did not correlate with the transgene copy numbers of the different lines (Table 1). An additional CEA mRNA species that comigrated with the 28S rRNA was seen in mouse 3077, but this was not further investigated.

The steady state protein levels in the feces, serum, and colon of transgenic mice have been determined using a CEA EIA and CEA ELISA and compared with the amounts found in humans (Table 1). These protein levels were approximately 10 times higher for transgenic line 2683 and 20 times higher for line 2682 in serum, and 2 times and 4 times higher, respectively, in feces, compared to the normal human values. CEA levels in mice colon extracts were also elevated, compared to normal human values. The sizes of the CEA protein extracted from mouse and human colon were compared by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, whereby CEA from mouse tissue ($M_r \approx 160{,}000$) was smaller than CEA from human colonic tissue ($M_r \approx 210{,}000$). We are presently investigating the reasons for this size discrepancy, which could possibly be due to differences in glycosylation.

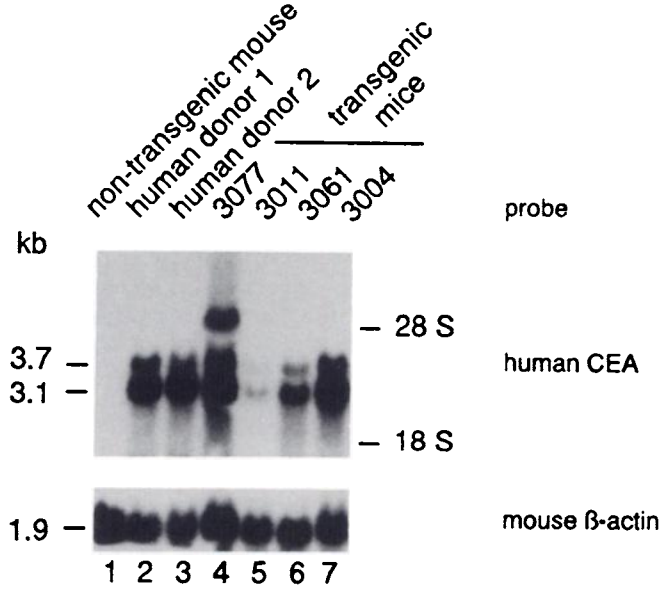

Fig. 3. Northern blot analysis of CEA expression in colons of different transgenic lines. *Top*, 10 μg of total RNA from colons of a nontransgenic mouse and two humans and total colonic RNA from transgenic mouse lines 2694 (mouse 3077), 2681 (mouse 3011), 2683 (mouse 3061), and 2682 (mouse 3004) were hybridized with a ^{32}P-labeled probe containing the full-length CEA cDNA. The sizes of the CEA transcripts are indicated to the left of the blot. In the right margin, the positions of the 28S and 18S rRNAs are shown. *Bottom*, after decay of the CEA probe the blot was rehybridized with a mouse β-actin probe.

Table 1 *CEA concentration in colonic tissue, feces, and serum of mice transgenic for CEA, compared to humans*

Species	Strain	Transgene copy number/ haploid genome[a]	CEA concentration[a] Colonic tissue (ng/mg of total protein)	Feces (ng/mg of total protein)	Serum (ng/ml)
Mouse	2681	2.6 ± 0.2 (n = 2)	ND[b]	ND	ND
	2682	1.6 ± 0.2 (n = 5)	1,350[c]	57,200 ± 19,100 (n = 9)	30.5 ± 12.7 (n = 6)
	2683	5.6 ± 0.6 (n = 4)	1,800[d]	28,400 ± 10,600 (n = 5)	14.0 ± 4.4 (n = 9)
	2694	8.5 ± 1.2 (n = 4)	ND	ND	ND
Human		NA[e]	108 ± 38 (n = 2)	13,800 ± 12,400 (n = 9)	1.5[f]

[a] Mean ± SD.
[b] ND, not determined.
[c] Colon extracts from three mice were pooled.
[d] Colon extract from one mouse.
[e] NA, not applicable.
[f] Value taken from the literature.

Immunohistochemical Analysis of CEA Expression in Adult Mice. We analyzed various mouse organs in the different lines (2682 and 2683 were studied in detail) by immunohistochemistry using the chimeric mAb cT84.66, to investigate in which tissues CEA is produced. As well as being specific for CEA, mAb cT84.66 has the additional advantage that endogenous mouse immunoglobulins do not interfere with immunostaining, because anti-human immunoglobulin antibodies can be used to detect the primary antibody. In mice transgenic for *CEA*, we detected CEA in those organs which expressed its RNA. In nontransgenic littermates, no staining was seen in any organs, as exemplified for colon (Fig. 4*B*). We found CEA in the epithelial cells along the crypts of transgenic mouse colon but it did not seem to be confined to the apical surface as in humans (Fig. 4*A*). Furthermore, CEA was present in the crypts but not in the villi of the small intestine (Fig. 4*C*), in the superficial zone of the gastric mucosa (Fig. 4*D*), on the squamous epithelial cells of the esophagus (Fig. 4*E*), and in the papillae filiformae of the tongue (Fig. 4*F*). In addition to the expression of CEA along the gastrointestinal tract, CEA was found in squamous epithelial cells of the trachea (Fig. 4*G*) and alveoli of the lung (Fig. 4*H*). No staining was seen in the liver (Fig. 4*I*) or various other organs (Table 2).

Immunohistochemical Analysis of CEA Expression During Mouse Development. We have analyzed the expression of CEA during mouse development, at embryonic days 8.5, 10.5, 12.5, 14.5, 16.5, and 19.5. CEA was first seen at embryonic day 10.5 in the primary trophoblast giant cells of the placenta that are in direct contact with the maternal decidua (Fig. 5, *A* and *B*). After embryonic day 16.5, CEA was no longer found in the placenta, which correlates with the disappearance of these giant cells (26). CEA could not be detected within the embryo until embryonic day 14.5, when the developing gut, trachea, lung, urethra, and nucleus pulposus of the vertebral column stained weakly. A stronger staining of these organs was found during progression of development (Fig. 5, *C-H*). CEA was also seen in squamous epithelia of the tongue and the nasal mucosa from embryonic day 16.5 onwards. All of these data are summarized in Table 2.

DISCUSSION

The spatiotemporal expression pattern of the human *CEA* gene in animals of the different mouse lines analyzed is essentially identical to that observed in humans. *CEA* gene expression has been reported in humans, at either the mRNA or protein level, in the colon, small intestine, stomach, esophagus, tongue, lung, testis, and cervix (11, 27–33). Tissues and organs that are CEA-negative in humans, *e.g.*, liver, kidney, salivary glands, and pancreas, are also negative in transgenic animals (11, 30). During human embryogenesis, CEA is first seen around the eighth week of development (1, 27, 28). This corresponds to embryonic day 14 of mouse development (34), where CEA can first be detected in the embryos of *CEA*-transgenic mice.

The transgene copy number and the CEA mRNA steady state levels in the colons of four transgenic lines analyzed in Fig. 3 do not closely correlate with the higher CEA steady state levels found in the colonic extracts, feces, and serum of these transgenic mice, compared to humans (Table 1). This indicates different post-translational regulation mechanisms or longer CEA turnover rates in the transgenic mice than in humans.

CEA is not expressed during early embryonic development in either humans or transgenic mice. In these mice, it first appears in the trophoblast giant cells of the placenta from embryonic day 10.5 until embryonic day 16.5, which correlates with a recent report of CEA in human syncytiotrophoblast cells (35). The primitive gut of mice develops around embryonic day 8, but CEA is first definitively found in the developing gut at embryonic day 14.5, so that it cannot play a role in organogenesis, as reported for other cell adhesion molecules, *e.g.*, cadherins (36); however, it could play a role in the conversion of the multilayered embryonic intestinal epithelia into the single-epithelial cell layer lining the mature gut. Interestingly, mouse BGP1 (former name, mmCGM1/2), which possibly represents the mouse CEA analogue, has a similar late onset of expression during embryogenesis in the intestine (37).

In spite of the relatively high levels of CEA in some of the transgenic mouse strains, no obvious phenotypic differences have been observed in comparison to their nontransgenic siblings. Similar results were observed for *CEA*-transgenic mice that ubiquitously express *CEA* under the control of the simian virus 40 early promoter (38).

The expression pattern of the transgene reported here implies that all regulatory elements for correct spatiotemporal expression are present in the human genomic DNA fragment used to generate the transgenic mice and that they are correctly recognized by murine *trans*-acting factors. This is especially noteworthy because no mouse *CEA* gene has yet been discovered, despite intensive searches. This suggests that the regulatory elements and transcription factors are strongly conserved during evolution. Furthermore, since the four mouse strains analyzed show identical expression patterns, the transgene activity is independent of the site of integration. This indicates the presence of sequences in the transferred human genomic DNA fragment which shield the *CEA* gene from the influences of regulatory elements close to the sites of transgene insertion.

Because the *CEA* transgene is mainly expressed in epithelial cells of the gastrointestinal tract, its regulatory elements could be valuable for studying tumorigenesis *in vivo*. These epithelial cells have an extremely rapid cellular turnover and are continuously regenerated from a multipotent stem cell in each crypt (reviewed in Ref. 39). In the colon they migrate from the base of the crypt to a hexagonally shaped

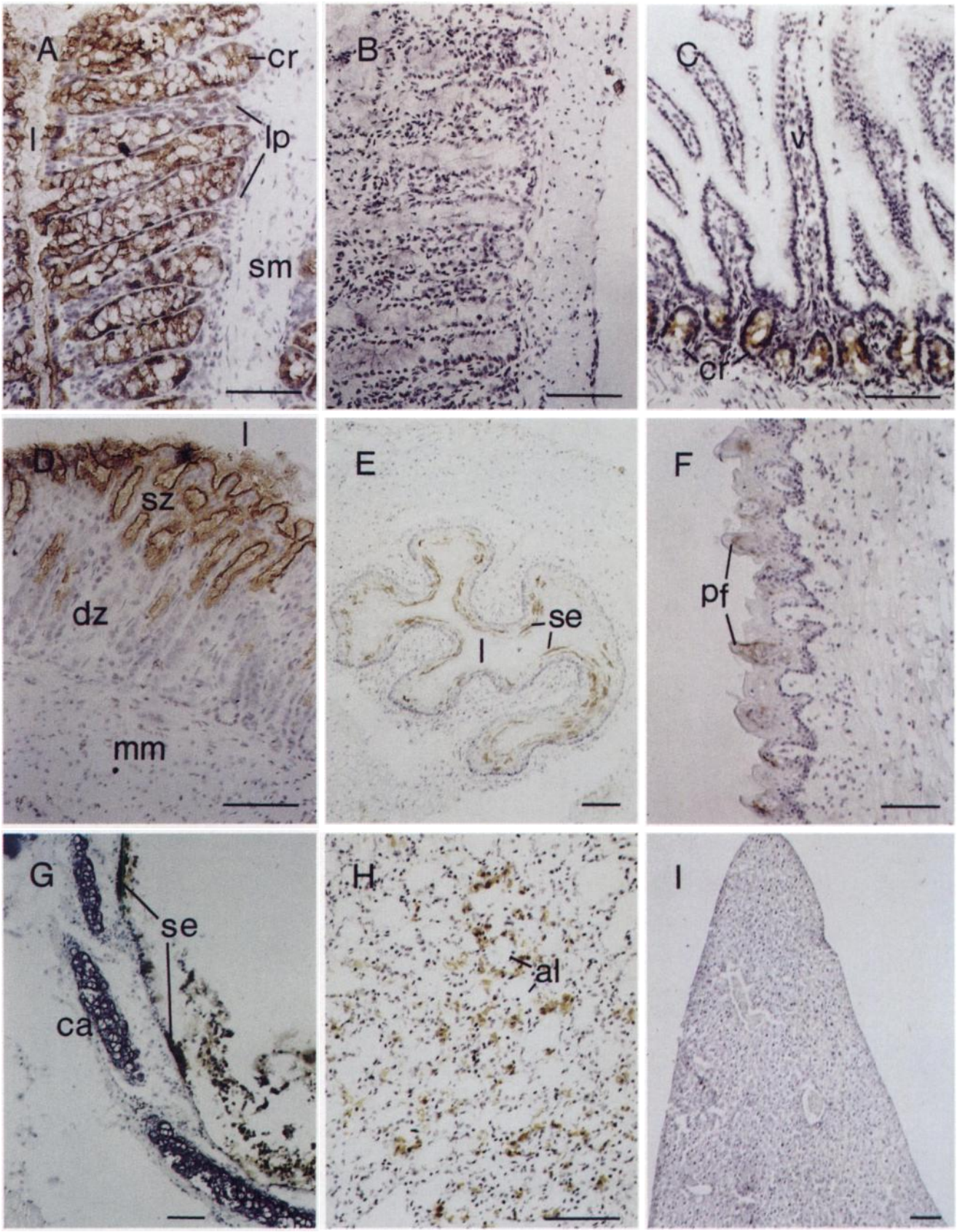

Fig. 4. Immunohistochemical analyses of human CEA in adult mice. Cryostat sections of organs from lines 2682, 2683, and 2694 were stained with the chimeric anti-CEA mAb T84.66 using the indirect peroxidase method. Similar staining patterns were obtained with all three transgenic lines. *A*, colon of transgenic mouse 3820 (line 2682); note apical staining of enterocytes (*lower left*); *B*, colon from a nontransgenic mouse; *C*, small intestine from transgenic mouse 3007 (line 2682); *D*, glandular region of the stomach (mouse 3079, line 2694); *E*, esophagus (mouse 128, line 2682); *F*, tongue (mouse 3007, line 2682); *G*, trachea (mouse 124, line 2683); *H*, lung (mouse 128, line 2682); *I*, liver (mouse 3079, line 2694). *al*, alveolae; *ca*, cartilage; *cr*, crypt; *dz*, deep zone of gastric foveolae; *l*, lumen; *lp*, lamina propria; *mm*, muscularis mucosae; *pf*, papillae filiformes; *se*, squamous epithelium; *sm*, submucosa; *sz*, superficial zone of gastric foveolae; *v*, villus. *Horizontal bar*, 100 μm.

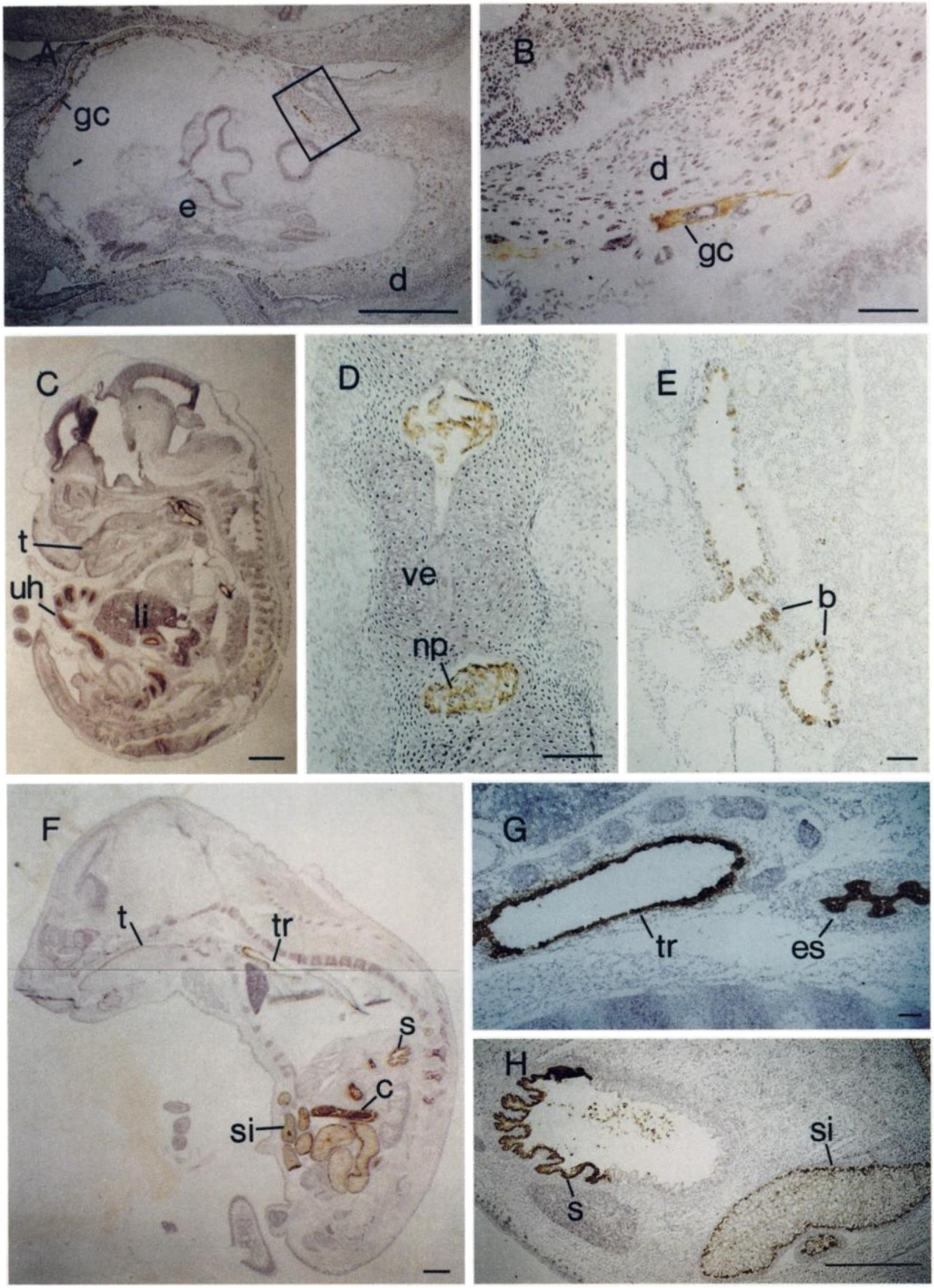

Fig. 5. Immunohistochemical analyses of human CEA during mouse embryogenesis. Cryostat sections of embryos (line 2683) were stained with the chimeric anti-CEA mAb T84.66 using the indirect peroxidase method. *A*, transverse section through mouse uterus with embryo at embryonic day 10.5, where staining is limited to the outer trophoblast layer; *B*, higher magnification of the region indicated in *A*, showing staining of a giant cell in close contact with maternal decidua cells; *C*, sagittal section of a whole embryo at embryonic day 16.5; *D*, vertebrae with nucleus pulposus stained in an embryonic day 16.5 embryo; *E*, lung from an embryo (embryonic day 19.5) with stained bronchiolar epithelium; *F*, sagittal section of a whole embryo at embryonic day 19.5; *G*, sagittal section of an embryonic day 19.5 embryo with staining in squamous epithelia of the trachea and esophagus; *H*, sagittal section of an embryonic day 19.5 embryo stained in the stomach and small intestine. *b*, bronchioli; *c*, colon; *d*, decidua; *e*, embryo; *es*, esophagus; *gc*, trophoblast giant cells; *li*, liver; *np*, nucleus pulposus; *s*, stomach; *si*, small intestine; *t*, tongue; *tr*, trachea; *uh*, umbilical hernia containing gut; *ve*, vertebra. *Horizontal bar*, 100 μm in *B*, *D*, *E*, and *G* and 1 mm in *A*, *C*, *F*, and *H*.

Table 2 *Expression pattern of human CEA during embryonic development and in adult transgenic mice (lines 2682 and 2683)*

Organ	Embryonic day 10.5 (−)[a]	12.5 (−)	14.5	16.5	19.5	Adult
Nasal mucosa			−	+	+	ND[b]
Tongue			−	+	+	+
Esophagus			+	+	+	+
Stomach			+	+	+	+
Small intestine			+	+	+	+
Colon/rectum			+	+	+	+
Nucleus pulposus			+	+	−	ND
Urethra			+	+	+	ND
Trachea/lung			+	+	+	+
Bile duct			ND	+	ND	ND
Liver			−	−	−	−
Heart			−	−	−	−
Kidney			−	−	−	−
Salivary glands			−	−	−	−
Pancreas			−	−	−	−
Thymus			−	−	−	−
Spleen			−	−	−	−
Brain			−	−	−	−
Placenta	+	+	+	+	−	NA[c]

[a] (−), although some of the organs cannot be distinguished at these stages of development, no staining whatsoever was seen within the embryo.
[b] ND, not determined.
[c] NA, not applicable.

cuff of surface epithelial cells and are subsequently exfoliated into the intestinal lumen. Since CEA protein is found down to the base of the crypts in the colon of the transgenic animals, the *CEA* gene is possibly active in the self-renewing and positionally fixed crypt stem cells. If this is the case, the *CEA* promoter could be used to direct expression of activated oncogenes or dominant negative mutants of tumor suppressor genes to intestinal stem cells, to study the genetic events during intestinal tumorigenesis. A similar approach has been attempted using the intestinal fatty acid-binding protein gene promoter to drive the expression of various oncogenes in the intestine. However, while dysplastic changes did occur, no tumor formation was observed in that system, which was probably due to the lack of promoter activity in the stem cells and the rapid exfoliation of the epithelial cells (40).

Finally, the *CEA*-transgenic mice offer an animal model system for optimizing tumor immunotherapy prior to clinical trials using CEA as a target antigen. To achieve this, adenomas or adenocarcinomas must first be induced in these transgenic animals, either chemically or by breeding with tumor-prone mice. If these tumors express CEA, this system would have a number of advantages over currently available animal models, *e.g.*, human tumors transplanted into athymic nude mice (41) or mice inoculated with syngeneic tumor cells transfected with a CEA cDNA (42). Firstly, the expression of CEA in normal tissues will allow investigations into the possible negative side effects of both active and passive immunotherapy. Secondly, *CEA*-transgenic animals are fully immunocompetent, in contrast to athymic nude mice. Thirdly, the induced CEA-expressing tumors have an authentic location, which would mirror the human situation more closely. Fourthly, assuming tolerance to CEA in these transgenic mice, this system may be used to optimize immunolocalization of tumors with CEA as a target antigen, since no masking of the xenoantigen by induced antibodies should occur. This has been a problem with the syngeneic transfectant approach (21). Although these transgenic mice exhibit relatively high serum levels of CEA, compared to healthy humans (Table 1), this should not be a problem for targeting tumors, because even very high CEA serum concentrations (500 ng/ml) do not prevent effective homing of anti-CEA antibodies through complex formation (43).

ACKNOWLEDGMENTS

The excellent technical assistance of M. Ditter and G. Sansig and animal caretaking by M-O. Schellinger is gratefully acknowledged. We would like to thank Drs. B. Christ and R. Kammerer for help in anatomical analyses of mouse fetuses, Dr. F. Grunert for the gift of antibodies for the CEA ELISA, and Dr. C. Bonifer for the mouse *Thy-1* probe. CEA EIA kits were generously provided by Hoffmann-La Roche (Basel, Switzerland).

REFERENCES

1. Shively, J. E., and Beatty, J. D. CEA-related antigens: molecular biology and clinical significance. Crit. Rev. Oncol. Hematol., *2:* 355–399, 1985.
2. Goldenberg, D. M. Cancer imaging with CEA antibodies: historical and current perspectives. Int. J. Biol. Markers, *7:* 160–166, 1992.
3. Beatty, J. D. Immunotherapy of colorectal cancer. Cancer (Phila.), *70* (Suppl. 5)*:* 1425–1433, 1992.
4. Khan, W. F., Teglund, S., Bremer, K., and Hammarström, S. The pregnancy-specific glycoprotein family of the immunoglobulin superfamily: identification of new members and estimation of family size. Genomics, *12:* 780–787, 1992.
5. Thompson, J. A., Grunert, F., and Zimmermann, W. The carcinoembryonic antigen gene family: molecular biology and clinical perspectives. J. Clin. Lab. Anal., *5:* 344–366, 1991.
6. Benchimol, S., Fuks, A., Jothy, S., Beauchemin, N., Shirota, K., and Stanners, C. P. Carcinoembryonic antigen, a human tumor marker, functions as an intercellular adhesion molecule. Cell, *57:* 327–334, 1989.
7. Oikawa, S., Inuzuka, C., Kuroki, M., Arakawa, F., Matsuoka, Y., Kosaki, G., and Nakazato, H. A specific heterotypic cell adhesion activity between members of carcinoembryonic antigen family, W272 and NCA, is mediated by N-domains. J. Biol. Chem., *266:* 7995–8001, 1991.
8. Kodelja, V., Lucas, K., Barnert, S., von Kleist, S., Thompson, J. A., and Zimmermann, W. Identification of a carcinoembryonic antigen gene family in the rat: analysis of the N-terminal domains reveals immunoglobulin-like, hypervariable regions. J. Biol. Chem., *264:* 6906–6912, 1989.
9. Rudert, F., Saunders, A. M., Rebstock, S., Thompson, J. A., and Zimmermann, W. Characterization of murine carcinoembryonic antigen gene family members. Mamm. Genome, *3:* 262–273, 1992.
10. McCuaig, K., Rosenberg, M., Nedellec, P., Turbide, C., and Beauchemin, N. Expression of the Bgp gene and characterization of mouse colon biliary glycoprotein isoforms. Gene, *127:* 173–183, 1993.
11. Nap, M., Mollgard, K., Burtin, P., and Fleuren, G. J. Immunohistochemistry of carcino-embryonic antigen in the embryo, fetus and adult. Tumor Biol., *9:* 145–153, 1988.
12. Stoffel, A., Neumaier, M., Gaida, F. J., Fenger, U., Drzeniek, Z., Haubeck, H. D., and Wagener, C. Monoclonal, anti-domain and anti-peptide antibodies assign the molecular weight 160,000 granulocyte membrane antigen of the CD66 cluster to a mRNA species encoded by the biliary glycoprotein gene, a member of the carcinoembryonic antigen gene family. J. Immunol., *150:* 4978–4984, 1993.
13. Lei, K. J., Sartwell, A. D., Pan, C. J., and Chou, J. Y. Cloning and expression of genes encoding human pregnancy-specific glycoproteins. J. Biol. Chem., *267:* 16371–16378, 1992.
14. Schrewe, H., Thompson, J., Bona, M., Hefta, L. J. F., Maruya, A., Hassauer, M., Shively, J. E., von Kleist, S., and Zimmermann, W. Cloning of the complete gene for the carcinoembryonic antigen: analysis of its promotor indicates a region conveying cell type-specific expression. Mol. Cell. Biol., *10:* 2738–2748, 1990.
15. Hefta, L. J. F., Schrewe, H., Thompson, J. A., Oikawa, S., Nakazato, H., and Shively, J. E. Expression of complementary DNA and genomic clones for carcinoembryonic antigen and nonspecific cross-reacting antigen in Chinese hamster ovary and mouse fibroblast cells and characterization of membrane expressed products. Cancer Res., *50:* 2397–2403, 1990.
16. Tautz, D., and Renz, M. An optimized freeze-squeeze method for the recovery of DNA fragments from agarose gels. Anal. Biochem., *132:* 14–19, 1983.
17. Botteri, F. M., van der Putten, H., Wong, D. F., Sauvage, C. A., and Evans, R. M. Unexpected thymic hyperplasia in transgenic mice harboring a neuronal promoter fused with simian virus 40 large T antigen. Mol. Cell. Biol., *7:* 3178–3184, 1987.
18. Sambrook, J. E., Fritsch, J., and Maniatis, T. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1989.
19. Grosveld, F., van Assendelft, G. B., Greaves, D. R., and Kollias, G. Position-independent, high-level expression of the human β-globin gene in transgenic mice. Cell, *51:* 975–985, 1987.
20. Chomczynski, P., and Sacchi, N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem., *162:* 156–159, 1987.
21. Pèlegrin, A., Terskikh, A., Hayoz, D., Chalandon, Y., Olsson, N-O., Folli, S., Buchegger, F., Kromer, B., Schwarz, K., Martin, M., Martin, F., and Mach, J-P. Human carcinoembryonic antigen cDNA expressed in rat carcinoma cells can function as a target antigen for tumor localization of antibodies in nude rats and as rejection antigen in syngeneic rats. Int. J. Cancer, *52:* 110–119, 1992.
22. Daniel, S., Nagel, G., Johnson, J., Lobo, F. M., Hirn, M., Jantscheff, P., Kuroki, M., von Kleist, S., and Grunert, F. Determination of the specificities of monoclonal antibodies recognizing members of the CEA family using a panel of transfectants. Int. J. Cancer, *55:* 303–310, 1993.
23. Alonso, S. A., Minty, A., Bourlet, Y., and Buckingham, M. Comparison of three actin-coding sequences in the mouse: evolutionary relationships between the actin

genes of warm-blooded vertebrates. J. Mol. Evol., *23:* 11–22, 1986.
24. Neumaier, M., Shively, L., Chen, F-S., Gaida, F-J., Ilgen, C., Paxton, R. J., Shively, J. E., and Riggs, A. D. Cloning of the genes for T84.66, an antibody that has a high specificty and affinity for carcinoembryonic antigen, and expression of chimeric human/mouse T84.66 genes in myeloma and Chinese hamster ovary cells. Cancer Res., *50:* 2128–2134, 1990.
25. Oikawa, S., Inuzuka, C., Kosaki, G., and Nakazato, H. Exon-intron organization of a gene for pregnancy-specific β_1-glycoprotein, a subfamily member of CEA family: implications for its characteristic repetitive domains and C-terminal sequences. Biochem. Biophys. Res. Commun., *156:* 68–77, 1988.
26. Kaufman, M. H. The Atlas of Mouse Development, pp. 476–477. London: Academic Press, 1992.
27. Wagener, C., Hain, F., Födisch, H-J., and Breuer, H. Localisation of carcinoembryonic antigen in embryonic and fetal human tissues. Histochemistry, *78:* 1–9, 1983.
28. von Kleist, S., Winkler, J., Migule, I., and Böhm, N. Carcinoembryonic antigen (CEA) expression in early embryogenesis: a study of the first trimester of gestation. Anticancer Res., *6:* 1265–1272, 1986.
29. Zimmermann, W., Ortlieb, B., Friedrich, R., and von Kleist, S. Isolation and characterization of cDNA clones encoding the human carcinoembryonic antigen reveal a highly conserved repeating structure. Proc. Natl. Acad. Sci. USA, *84:* 2960–2964, 1987.
30. Zoubir, F., Zeromski, J., Sikora, J., Szmeja, J., and Hedin, A. Tumor specificity of monoclonal antibodies to carcinoembryonic antigen. Tumor Biol., *11:* 5–19, 1990.
31. Kim, J., Kaye, F. J., Henslee, J. G., Shively, J. E., Park, J-G., Lai, S-L., Linnoila, R. I., Mulshine, J. L., and Gazdar, A. F. Expression of carcinoembryonic antigen and related genes in lung and gastrointestinal cancers. Int. J. Cancer, *52:* 718–725, 1992.
32. Sanders, D. S. A., Stocks, S. C., Milne, D. M., Milne, G. A., Hopwood, D., and Kerr, M. A. Membranous expression of carcinoembryonic antigen (CEA) in the normal cervical squamous mucosa. J. Pathol., *167:* 77–82, 1992.
33. Beatty, B. G., Paxton, R. J., Sheibani, K., Duda, R. B., Williams, L. E., Shively, J. E., and Beatty, J. D. Testis imaging with ^{111}In-labeled anticarcinoembryonic antigen monoclonal antibody: identification of carcinoembryonic antigen in normal germ cells. Cancer Res., *46:* 6503–6508, 1986.
34. Rugh, R. The Mouse: Its Reproduction and Development. Oxford, UK: Oxford University Press, 1990.
35. Babury, R. A., and Moscovic, E. A. Selective immunolabeling of early gestational cytotrophoblast and its neoplastic counterpart by the monoclonal antibody Ber-EP4. Histol. Histopathol., *8:* 323–328, 1993.
36. Edelman, G. M. Cell adhesion molecules in the regulation of animal form and tissue pattern. Annu. Rev. Cell Biol., *2:* 81–116, 1986.
37. Huang, J. Q., Turbide, C., Daniels, E., Jothy, S., and Beauchemin, N. Spatiotemporal expression of murine carcinoembryonic antigen (CEA) gene family members during mouse embryogenesis. Development, *110:* 573–588, 1990.
38. Hasegawa, T., Isobe, K., Tsuchiya, Y., Oikawa, S., Nakazato, H., Ikezawa, H., Nakashima, I., and Shimokata, K. Establishment and characterization of human carcinoembryonic antigen transgenic mice. Br. J. Cancer, *64:* 10–14, 1991.
39. Gordon, J. I., Schmidt, G. H., and Roth, K. A. Studies of intestinal stem cells using normal, chimeric, and transgenic mice. FASEB J., *6:* 3039–3050, 1992.
40. Kim, S., Roth, K., Moser, A., and Gordon, J. Transgenic mouse models that explore the multistep hypothesis of intestinal neoplasia. J. Cell Biol., *123:* 877–893, 1993.
41. Buchegger, F., Vacca, A., Carrel, S., Schreyer, M., and Mach, J-P. Radioimmunotherapy of human colon carcinoma by [^{131}I]-labelled monoclonal anti-CEA antibodies in a nude mouse model. Int. J. Cancer, *41:* 127–134, 1988.
42. Kantor, J., Irvine, K., Abrams, S., Kaufman, H., DiPietro, J., and Schlom, J. Antitumor activity and immune responses induced by a recombinant vaccinia-carcinoembryonic antigen (CEA) vaccine. J. Natl. Cancer Inst., *84:* 1084–1091, 1992.
43. Sharkey, R. M., Goldenberg, D. M., Murthy, S., Pinsky, H., Vagg, R., Pawlyk, D., Siegel, J. A., Wong, G. Y., Gascon, P., Izon, D. O., Yezza, M., Burger, K., Swayne, L. C., Pinsky, C. M., and Hansen, H. J. Clinical evaluation of tumor targeting with high-affinity, anticarcinoembryonic-antigen-specific, murine monoclonal antibody, MN-14. Cancer (Phila.), *71:* 2082–2096, 1993.