Studies on the immunogenicity of hCEA in a transgenic mouse model

Abstract  Background and aims: Immunization protocols in mice have shown that the tumor-associated antigen hCEA could be a target for active immunization; however, human CEA is foreign to mice. Success may depend in part on a simple anti-xenoresponse. Using hCEA-transfected syngeneic tumor cells in hCEA-transgenic mice should bypass this problem and allow testing for new vaccination strategies.  Materials and methods: We established a hCEA transgenic model of the haplotype H2d, which may differ from other haplotypes in cytokine production and in effectiveness of antigen presentation, and tested two vaccination protocols in wild-type and transgenic mice.  Results: Syngeneic wild-type mice built up an immune response with high antibody titers; only 65% of animals developed solid tumors after tumor challenge. In contrast, hCEA-transgenic mice developed no antibody response and accepted the tumor in more than 90% of cases, thus demonstrating the role of human CEA as a foreign antigen. Accordingly, active immunization using tumor lysate or lymphocytes loaded with hCEA resulted in a CTL response and tumor-rejection in up to 80% of wild-type mice. hCEA-transgenic mice could be induced with both immunization protocols to build up a CTL response, although the number of CTL were much lower and the cytotoxic response weaker than in wild-type mice. In vivo hCEA-transgenic mice rejected hCEA-positive tumors only after immunization with the tumor lysate in about 60% whereas there was no rejection of tumors after immunization with the human hCEA-loaded autologous lymphocytes.  Conclusion: The findings clearly show the importance of transgenic models when testing the effects of immunization towards human tumor associated antigens such as hCEA because results differ in wild-type and transgenic mice.

Keywords  Carcinoembryonic antigen · CEA transgenic mice · Cancer vaccine · Cross-priming

Introduction

Active immunization to tumor antigens is one of the major goals in cancer therapy. The recent establishment of cytotoxic T-lymphocyte (CTL) lines from patients which clearly recognize tumor cells and the characterization of tumor antigens using these CTL as a tool support the concept that active immunization to tumor antigens should expand the therapeutic repertoire [1, 2, 3, 4, 5].

Although CTL can be easily generated in animal models, progress in clinical application seems rather slow. One explanation for the different results might be that in mice models human or even bacterial genes with no apparent homology to murine genes have been introduced into animal cells. The product of these foreign
genes may induce an allosresponse in these experimental models. Therefore developing a clinically useful protocol confronts the problems of choosing an attractive human tumor antigen and overcoming species barriers in test systems. This can be accomplished by using a well defined tumor antigen in transgenic animals.

Of all tumor antigens described, tumor-associated antigens are best described and are therefore accessible as good candidates for immunization. Carcinoembryonic antigen (CEA) is such a well defined transmembrane protein expressed early in life on epithelial cells [6, 7]. Later CEA is extensively expressed in the vast majority of colorectal, gastric, and pancreatic adenocarcinomas as well as in 50% of breast cancers and 70% of non-small-cell lung carcinomas. This high expression on tumors and the limited expression on normal tissue makes it an attractive candidate for immunization.

There are several possible immunization protocols and routes available. For CEA a vaccinia-based immunization has been shown to induce not only a CTL response but also tumor regression in a mouse model [8, 9]. Results in humans have shown a weak CTL response after immunization with the vaccinia system [10, 11, 12] and with peptide and adjuvant immunization protocols [13]. The setbacks noted above may account for this difference since the animal experiments have been performed in mice, where CEA is absent, and only distantly related proteins exist [14]. We therefore established a model system using hCEA-transgenic mice and hCEA-transfected tumor cells. In this model we tested tumor growth characteristics and immunization protocols and compared the results with those in nontransgenic litters.

Materials and methods

Mice

DBA/2 were purchased from Charles River (Sulzfeld, Germany). Animals were kept under standard conditions according to governmental guidelines. hCEA transgenic mice were generated as described elsewhere [14]. Transgenic mice (H-2b/H2d chimeras) were back-crossed to DBA/2 (H-2d) mice for at least ten generations. Transgenic animals were identified by the analysis of tail-DNA by PCR or the measurement of hCEA in feces [14].

hCEA-transfection of cells

The mouse leukemia cell line L1210 (American Type Culture Collection, Rockville Md. USA) was transfected with an expression vector (pReCMV, Invitrogen, San Diego, Calif., USA) containing the full-length human CEA cDNA by electroporation [15]. Transfected cells were selected in medium containing G418 (1 mg/ml, Gibco BRL, Eggenstein, Germany). hCEA-expressing L1210 (L5) were expanded and maintained in complete RPMI 1640 medium supplemented with 10% fetal calf serum and G418 at a concentration of 500–750 µg/ml. L5 were regularly tested for hCEA expression by fluorescence-activated cell sorter (FACS) analysis (FACSort, BD, Heidelberg, Germany) using a polyclonal antibody to hCEA (Dakopatts, Hamburg, Germany).

Induction of tumor growth

DBA/2 and transgenic mice were injected subcutaneously with 5×10^6 L5 or 5×10^6 L1210 tumor cells in 100 µl Ca/Mg-free Hank’s solution. The tumor size was monitored every second day using a microlipper, and individual tumor volumes were calculated using the following formula: 0.875πW×Hx×B. The mice were killed after 21 days, when the tumors reached a size of approximately 5000 mm³ in control animals. Serum was taken, the tumors were frozen, and a single-cell suspension of lymphocytes from the spleen was prepared.

Vaccination protocol

DBA/2 and transgenic mice were immunized with 10⁶ lysed L5 cells and wild-type L1210 control cells diluted in 500 µl complete Freund’s adjuvant (CFA). Of this solution 100 µl was injected intraperitoneally. In a separate set of experiments mice were immunized by intravenous injection of splenic lymphocytes hypertonically loaded with hCEA, as described elsewhere [16]. Briefly, 5×10⁹ Ficoll separated spleen lymphocytes were incubated with 2 mg/ml hCEA in a total volume of 0.5 ml hypertonic solution (0.5 M sucrose, 10% polyethylene glycol 1000, 10 mM hydroxyethylpiperazine ethanesulfonic acid in RPMI 1640) for 10 min at 37°C. This suspension was diluted with 15 ml hypotonic RPMI (60%) and left for 2 min at 37°C. Cells were then washed and used for intravenous injection. Loaded cells (1×10⁶) were slowly injected via the tail vein. Ten days after immunization the animals were either killed to isolate spleen lymphocytes or challenged in vivo with 5×10⁵ L5 or 5×10⁵ L1210 control cells subcutaneously. Isolated spleen lymphocytes were restimulated in vitro for 5 days with irradiated L5 (200 Gy) and 20 U/ml recombinant interleukin 2 (Boehringer-Mannheim, Mannheim, Germany).

Detection of hCEA antibodies

Antibodies to hCEA were measured in serum samples by enzyme-linked immunosorbent assay. Briefly, microtiter plates were coated overnight at 4°C with 2 µg/ml purified hCEA (BioGenes, Berlin, Germany). After blocking with 10% fetal calf serum in phosphate-buffered solution plates were incubated with sample dilutions (10⁻³ and 10⁻⁴) or an anti-hcea monoclonal antibody (6D7) as control for 1 h at room temperature. After washing bound immunoglobulin were detected with peroxidase-labeled anti-mouse IgG antibodies (Dakopatts) and ortho-phenylenediamine (Sigma, Deisenhofen, Germany) as substrate. Absorbance was measured at 490 nm on an automatic enzyme-linked immunosorbent assay reader (SLT, Crailsheim, Germany).

Chromium release assay

L5 or L1210 cells (1×10⁶) were labeled with 100 µCi ⁵¹Cr-sodium at 37°C for 60 min. Following washing 1×10⁴ labeled target cells and serial dilutions of effector cells were incubated in 200 µl RP10 in round-bottom microtiter plates in a humidified incubator at 37°C for 4 h. Thereafter the supernatant was collected by a filter device (Skatron, Norway) and the radioactivity measured on a gamma-counter. Specific lysis was calculated according to a standard method. Spontaneous release was always less than 25% of the maximal release induced by detergent lysis.

Polymerase chain reaction

Tail DNA and RNA were isolated according to a standard protocol. Amplification was performed on a Perkin-Elmer thermocycler using specific primers for human hCEA reported in the literature [17].