

Carcinoembryonic Antigen Transgenic Mouse Models for Immunotherapy and Development of Cancer Vaccines

UNIT 20.8

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ABSTRACT

The goal of cancer therapy remains as the long-term eradication of tumor cells without adverse effects on normal tissue. Conventional approaches utilizing chemotherapy and radiotherapy are limited by both their toxicity and lack of specificity. In recent years, investigators have carried out several studies designed to evaluate whether human tumor-associated antigens (TAAs) can be exploited as targets for immunotherapy, specifically for human cancer vaccine development. A major limitation in immunotherapy studies of human cancer is the general lack of appropriate preclinical models. Clinical studies can be difficult to implement, particularly when a clear understanding of the potential efficacy, limitation, and safety of an immunotherapeutic strategy is not available from relevant animal investigations. However, mice carrying a transgene for a human tumor self-antigen may provide a more acceptable experimental model in which knowledge about immunotherapeutic strategies aiming at the TAA of interest can be enhanced prior to initiating clinical trials. Since the different strategies in experimental immunotherapy of cancer have been directed to activate different immune system components, a variety of transgenic mouse models have been generated expressing either TAA, human leukocyte antigen (HLA), oncogene, or immune effector cell molecules. These models may serve as an excellent platform for the identification of novel targets for immunotherapy as well as to evaluate the efficacy of targeted therapies and will lead to the development of clinical trials for cancer patients. In this unit, a brief overview of the generation and study of different vaccine approaches in carcinoembryonic antigen (CEA) transgenic mouse models and the experimental findings in mouse models that spontaneously develop gastrointestinal tumors and express the CEA transgene is provided. *Curr. Protoc. Immunol.* 80:20.8.1-20.8.12. © 2008 by John Wiley & Sons, Inc.

Keywords: immunotherapy • cancer • vaccines • transgenic mouse model • carcinoembryonic antigen

INTRODUCTION

Cancer is an age-related disease and with the graying of society there is an increasing need to optimize cancer management and therapy for application in elderly patients. Although there are reports in the success of curing non-metastatic cancer (Donaldson et al., 1999; Morris and Bosl, 1999), most forms of metastatic cancer are incurable in the long run with conventional treatments such as surgery, radiotherapy, and chemotherapy. Cancer vaccines that can be applied in both prevention and therapy are potentially less toxic than chemotherapy or radiation and could, therefore, be especially suitable for older cancer patients. Since the first description of a human

TAA recognized by cytotoxic T cells (van der Bruggen et al., 1991), advances in understanding the nature of tumor-specific immune responses and mechanisms of tolerance induction have encouraged researchers and clinicians to develop a more defined approach to immune-mediated therapies. Current immunotherapeutic approaches are many and varied with respect to target specificities, effector mechanisms, mode of administration, and prospects for translation into clinical practice.

Targeted immunotherapeutic approach of cancer depends on the assumption that a tumor differs from normal tissues by the presence of tumor antigens. Tumor antigens can be divided into two main categories. The first group

**Animal Models
for Tumor
Immunology**

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Current Protocols in Immunology 20.8.1-20.8.12, February 2008

Published online February 2008 in Wiley Interscience (www.interscience.wiley.com).

DOI: 10.1002/0471142735.im2008s80

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Supplement 80

is known as tumor-specific antigens (TSAs), and the genes encoding TSA are present only in tumor cells and not in normal tissues. The second group called tumor-associated antigens (TAAs), are expressed at elevated levels in tumor cells but are also present in normal cells in trace amounts (Herlyn and Birebent, 1999). Theoretically, the presence of TSA or TAA would allow tumor cells to induce an immune response involving different effector cells, cytokines, growth factors, and antibodies. However, most tumors have developed mechanisms to escape immune surveillance by down regulation of MHC and/or costimulatory molecules (Garrido et al., 1997). Upregulation of molecules that induce anergy and/or apoptosis in the attacking immune effector cells is also a common feature of tumor cells (Gilboa, 1999).

Animal models have played a critical role in establishing basic paradigms of tumor immunology as they provide an *in vivo* milieu that cannot be reproduced *in vitro*. In the last decade, different strategies have been developed for experimental immunotherapy of cancer. These immunotherapeutic strategies have been applied either in the form of active immunotherapy using irradiated tumor cells, gene-modified tumor cells, plasmids or viral vectors encoding tumor antigens, dendritic cells (DCs) pulsed with tumor antigens or in the form of passive immunotherapy using tumor-specific antibodies, and/or cytokines, adoptive transfer of immune effector cells. Tolerance induction to specific tumor-antigens is a major obstacle for effective immune responses to tumor. A number of animal models have proven to be of great value, although more

Table 20.8.1 Selected Transgenic Mouse Models^a

Model	Antigen	Transgene	Reference
TAA	CEA	SV-40-CEAcDNA-SV40	Hasegawa et al. (1991)
		CEA genomic sequences	Eades-Perner et al. (1994); Clarke et al. (1998)
		CEA family gene cluster	Chan and Stanners (2004)
	CEA/MIN	CEA × APC mutated	Thompson et al. (1997); Wilkinson et al. (2001)
	CEA/A2K ^b	CEA × HLA-A2.1/K ^b	Zhou et al. (2004); Saha et al. (2007)
	PSA	PSA genomic sequences	Wei et al. (1997, 1998)
	MUC1	MUC1 genomic sequences	Rowse et al. (1998)
HLA	P1A	H-2K ^b -P1AcDNA-β-globin	Brandle et al. (1998)
	HLA-A2.1/K ^b		Vitiello et al. (1991)
	HLA-A2.1		Yu et al. (1997)
	HLA-A2.1/p53 ^{-/-}		McCarty et al. (1998)
	HLA-A2.1/K ^b /CD8 ⁺		Lustgarten et al. (1997)
Oncogene	SV40T	Antithrombin III-SV40T	Romieu et al. (1997)
	Tyr-SV40T		Anand et al. (1994)
	PyMidT	MMTV-PyMidTcDNA	Guy et al. (1992)
	ErbB-2	ErbB-2 cDNA	Piechocki et al. (2003)
IECM	TCR	L ^d -TCR; 2C	Sha et al. (1988)
		2C/Rag2 ^{-/-}	Manning et al. (1997)
		2C/Rag2 ^{-/-} /CD28 ^{-/-}	Fallarino et al. (1998)
	B7-1	RIP-B7-1/RIP-IL-2	Allison et al. (1992)
	FcR	FcγRIA genomic sequences	Heijnen and Van de Winkel (1995)

^aTAA, tumor-associated antigen; HLA, human leukocyte antigen; IECM, immune effector cell molecule.

relevant immunocompetent animal models resembling human diseases are needed. The first transgenic mice were generated in 1982 (Palmiter et al., 1982) and since then transgenic animal models have been extensively used to investigate important mechanisms underlying a variety of diseases including cancer. To evaluate tumor immunotherapeutic strategies, transgenic mice have been generated, which express TAAs, HLAs, oncogenes, and human immune effector cell molecules (Table 20.8.1).

CEA TRANSGENIC MICE

CEA is a 180-kDa cell-surface oncofetal glycoprotein and belongs to a family of 29 genes that is part of the immunoglobulin gene superfamily (Hammarstrom et al., 1998). Although its presence in normal tissue is mainly limited to the large intestine, it is overexpressed in most gastrointestinal malignancies, lung cancer, breast cancer, and thyroid cancer (Thompson et al., 1991). 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. Circulating CEA can be detected in the serum of patients with CEA-positive tumors and has been extensively used for disease staging and as an indicator of residual disease and/or tumor recurrences in these cancer patients (Shively and Beatty, 1985; Horig et al., 2000). Due to its unique expression pattern, CEA has been targeted as a potential TAA to induce CEA-specific antibodies, CD4⁺ T helper cells and CD8⁺ CTLs to treat CEA⁺ tumors (Berinstein, 2002).

To evaluate vaccines directed against CEA as a self-antigen, a useful animal model was required to elicit CEA-specific immune responses, as well as to identify any adverse autoimmune reactions. However, until ~15 years ago, using experimental mouse models to study different therapeutic interventions directed at CEA had been impossible since no CEA homolog had been identified in either mice or rat. Since then several mouse models expressing human CEA as a transgene have been developed and used extensively to study the generation of CEA-specific host immune responses and antitumor immunity. To date, at least four different mouse models expressing human CEA as a transgene have been developed. The initial CEA transgenic mouse line used the full-length CEA cDNA containing the SV40 early promoter and CEA was expressed in all tissues examined, including the

brain, thymus, lung, spleen, liver, kidney, and colon (Hasegawa et al., 1991). However, some cellular specificity of expression was evident because only the epithelial cells in the lung and colon were positive as shown by immunohistochemical staining. The next two mouse models (Eades-Perner et al., 1994; Clarke et al., 1998) used the complete CEA gene, including the flanking 33-kb regulatory elements to generate CEA transgenic mice. Although both groups used almost the same mouse strain, different CEA expression patterns were observed. CEA protein could be detected in different tissues including tongue, esophagus, stomach, small intestine, cecum, colon, and trachea, and low levels in lung, testis, and uterus in CEA transgenic mice (Eades-Perner et al., 1994). The CEA levels in the colon, serum, and feces were higher than the concentrations found in humans by 10-fold, 9- to 20-fold, and 2- to 4-fold, respectively. Furthermore, the CEA isolated from transgenic mouse tissue was smaller (~160 kDa) than the CEA isolated from human colonic tissue (~200 kDa) and the reason for this discrepancy is still unknown.

In the CEA transgenic mice generated by Clarke et al., CEA was only detected by immunohistochemistry in the large intestine, cecum, and stomach, more closely approximating the adult human CEA expression pattern (Primus et al., 1981). Serum CEA levels could not be measured under normal circumstances and the CEA concentration in feces of transgenic mice was 100-fold lower than found in humans. Also, CEA isolated from transgenic mice colon and CEA purified from human colonic adenocarcinomas were of similar size, indicating that the CEA molecule was processed normally. The differences in CEA expression patterns in these two CEA transgenic mouse models provided the opportunity to determine whether the degree of immune tolerance might impact the ability of CEA-based vaccine to induce CEA specific immune responses. The most recent mouse model was developed using a 187-kb human bacterial artificial chromosome that contained part of the human CEA family gene cluster, including complete CEA, CEA-related cell adhesion molecule-3 (CEACAM3), CEACAM6, and CEA-CAM7 sequences (Chan and Stanners, 2004). This model has provided the opportunity to target other CEACAMs in vaccine-based protocols and better determine whether autoreactive T cells may mediate autoimmunity directed at normal tissues that expressed the different CEACAMs.

CEA VACCINES

Gene-based vaccines

DNA vaccines encoding a variety of antigens have been shown to induce cell-mediated immune responses resulting in tumor-protective immunity in several animal models of cancer. A number of available strategies, which improve the potency of DNA vaccines include vector modifications, which enhance antigen expression and target the protein expressed to a particular cellular location, together with inclusion of immunostimulatory sequences (Rodriguez et al., 1997) and improvements in DNA delivery systems (Schlesinger and Dubensky, 1999) or use of adjuvants (Agadjanyan et al., 1999). Several recent efforts focused on DNA-based cancer vaccines designed to overcome peripheral T-cell tolerance against tumor self-antigens including CEA. In this regard, following in vivo priming with CEA-transfected fibroblasts, anti-CEA CD8⁺ T cells could be elicited in CEA transgenic mice that were tolerant to CEA in the CD4⁺ T-cell compartment (Mizobata et al., 2000). An oral vaccine containing the entire CEA gene carried by an attenuated strain of *Salmonella typhimurium*, effectively protected CEA transgenic mice against a lethal challenge of murine colon carcinoma cells, MC-38, stably transduced with CEA. This protective effect was primarily induced by MHC class I-restricted CD8⁺ T cells and could be further amplified by a recombinant antibody-IL-2 fusion protein (Xiang et al., 2001a,b). The peripheral T cell tolerance to CEA was also overcome by a DNA vaccine containing the entire gene coding for CEA when delivered intramuscularly by a DNA delivery system of cationic poly(lactide-coglycolide) (PLG) microparticles boosted by GM-CSF (Luo et al., 2003). The tumor-protective immunity was due to activation of CD8⁺ T cells and secretion of pro-inflammatory cytokines such as IFN- γ , TNF- α , and IL-2.

Recombinant poxviruses have been engineered to express a wide variety of foreign antigens and have been studied by several laboratories as candidates for active immunization. Vaccinia virus, a poxvirus, is an attractive platform for a vaccine as it accepts large inserts of foreign genes, has a wide host range and can induce host immune response to the inserted gene product. In several CEA-based DNA vaccines, the use of replication-competent recombinant vaccinia and replication-defective recombinant fowlpox vectors containing a

triad of T-cell co-stimulatory molecules, B7.1, ICAM-1, and LFA-3 (TRICOM) resulted in the induction of CEA-specific T cell responses and antitumor immunity in CEA transgenic mice (Hodge et al., 2003a,b; Chakraborty et al., 2004; Kudo-Saito et al., 2005).

A recent study conducted by Ojima and colleagues (Ojima et al., 2006) employed a vaccination strategy to determine whether peripheral T cell tolerance to CEA could be overcome by vaccinating genetically modified DCs that simultaneously express CEA, granulocyte macrophage colony-stimulating factor (GM-CSF) and interleukin 12 (IL-12) in CEA transgenic mice. DCs adenovirally transduced with CEA and T helper-type 1 cytokine gene was reported to be very effective as the majority of tumor-bearing mice showed a complete eradication of CEA⁺ tumors. This antitumor immune response was mediated by coordinating effects of NK cells, CD8⁺ CTLs, and CD4⁺ helper T cells.

Plasmid DNA vaccine is a safe and promising method for genetic vaccination and have been used against various pathogens (Gurunathan et al., 2000), but the immune responses induced by DNA vaccines have been relatively weak compared to conventional vaccines. One reason for this limited efficacy is that in larger animals the level of protein expression might be insufficient to elicit strong immune responses. The enhancement of immune responses is particularly relevant for cancer vaccines because of the limited immunogenicity of tumor antigens and the need to overcome tolerance. The immunogenic properties of plasmid DNA and adenovirus vectors encoding CEA fused at its C terminus to a panel of immunoenhancing proteins were analyzed in CEA transgenic mice (Facciabene et al., 2006). Vectors encoding CEA fused to the minimized domain of tetanus toxin fragment C (DOM) could break tolerance to CEA and elicited significant CEA-specific antibody and CD8⁺ T cell-mediated response. Also, mice immunized with plasmid DNA and adenovirus vectors expressing CEA-DOM were protected from tumor growth after challenge with MC-38-CEA tumor cells. In another study, the use of plasmid DNA encoding CEA as an oral vaccine was able to induce CEA-specific CTL responses in CEA transgenic mice (Huang et al., 2005). CEA⁺ colorectal tumors are mainly located on the gastrointestinal mucosal surface. Ideally, CEA-specific CTLs induced by a CEA vaccine should be able to localize in the local

mucosal area and efficiently lyse neighboring CEA⁺ tumor cells. In that respect, this study is particularly important as Huang and colleagues were able to induce both mucosal and systemic CTL responses and also in a tumor prevention model, the growth of CEA⁺ tumors was significantly delayed in immunized mice.

The use of minigene vaccines is another promising approach because of the ease of synthesis and manipulation of these vaccines. In contrast to whole-gene vaccines, minigene vaccines can induce immune responses against specific antigen epitopes while avoiding the interference of irrelevant antigen epitopes. Recently, Zhou et al. (2004) have demonstrated that peripheral T cell tolerance against the CEA self-antigen could be broken by an oral DNA minigene vaccine consisting of CTL peptide of CEA in a more relevant mouse model transgenic for both CEA and HLA-A2. Of the two different HLA-A2-restricted epitopes used, CEA₆₉₁ (IMIGVLGV) peptide was more effective than CAP1-6D (YLSGADLNL) peptide as a DNA minigene in a bacterial carrier system for the induction of CTL response, which could specifically lyse CEA-A2Kb-transduced MC-38 colon carcinoma cells in vitro and protect mice from a challenge by these tumor cells in vivo.

Antibody-based vaccines

Another attractive approach of antitumor therapy is based on the induction of tumor-specific humoral immune responses. Monoclonal antibodies (mAbs) have been used to treat malignancies in humans with varying degrees of success and have emerged as an important therapeutic modality for cancer. Several therapeutic antibodies have been designed to exert their effect when coupled to immunotoxins, drugs, or radioisotopes (Riethmuller et al., 1998). A number of studies have been reported in which monoclonal anti-CEA antibodies have been used for antigen-specific passive immunotherapy in cancer patients (Abe et al., 2002; Behr et al., 2002). To improve the efficacy of the antitumor response, antibodies have also been fused to cytokines or growth factors including IL-2, IL-6, or GM-CSF (Tao and Levy, 1993; Penichet and Morrison, 2001; Reinartz et al., 2003). In recent years, several studies were designed to improve the potency of the antibody based immunotherapeutic strategy against animal tumor model of colon cancer in CEA transgenic mice.

Xu et al. (2000) performed a study for the generation of a genetically engineered biva-

lent single-gene-encoded anti-CEA antibody and its IL-2 fusion protein derivative for selective tumor targeting of the cytokine. The therapeutic efficacy of the anti-CEA-IL-2 fusion protein was evaluated in CEA-expressing colon carcinoma. The intraperitoneal administration of the fusion protein was effective for tumor growth inhibition, whereas injection of anti-CEA antibody alone or a mixture of antibody plus IL-2 could not inhibit the growth of CEA⁺ tumors. The therapeutic effects of the anti-CEA-IL-2 fusion protein were improved by intravenous injection and extending the duration of treatment. However, tumor cures were not observed. The ineffectiveness of the anti-CEA antibody alone was not surprising because CEA-specific monoclonal antibodies used were poor mediators of antibody dependent cellular cytotoxicity (Koga et al., 1990). Also, the antitumor properties of the fusion protein may have been limited by its faster egress from the tumor possibly caused in part by its higher κ_{off} as suggested by the authors.

In contrast to passive immunotherapy where large doses of preformed antitumor antibodies or T cells with predetermined specificity are infused, active specific immunotherapy through vaccines would aim to activate the immune system of the host to eliminate the tumors, as well as to induce a memory response against future tumor challenge. The use of anti-idiotypic (Id) antibodies as vaccines to stimulate antitumor immunity is one of several promising immunologic approaches to the therapy of cancer. The network hypothesis (Jerne, 1974) offers an elegant concept for developing vaccines, which is not based on the conventional approach of using nominal antigen. These so-called anti-Id vaccines or internal image antigen vaccines take advantage of the fact that the repertoire of external or nominal antigens is mimicked by Id structures on immunoglobulins and possibly on receptors and products of T cells as well. Thus, with this approach, Id-based vaccines do not contain nominal antigen or its fragments. This excludes the possibility that Id vaccines would have the same undesired side effects, which are sometimes associated with conventional antigen vaccines. Also, the predictability of the fine specificities of vaccine-induced immune responses to tumors is higher for anti-Id vaccines than for antigen vaccines.

Several monoclonal anti-Id antibodies, which mimic distinct human TAAs have been developed and characterized by the authors and others (reviewed in

Bhattacharya-Chatterjee et al., 2002). A number of anti-Id antibodies mimicking CEA have also been tested in the clinic and demonstrated encouraging results (Somasundaram et al., 1995; Foon et al., 1997, 1999; Durrant et al., 2000). Although several clinical studies have shown enhanced survival of patients receiving anti-Id vaccines, the outcome of these multi-center anti-CEA immunotherapeutic strategies in patients may or may not generate new insights and these trials emphasize the need for more realistic preclinical information obtained from animal models that express CEA as a self antigen.

The use of internal image anti-Id antibody vaccines mimicking TAAs aims to elicit effective immune responses that will be capable to eradicate tumor cells and prevent tumor recurrence. However, TAAs like the human CEA are self-antigens against which clinically relevant humoral or cellular immune responses are not easily achieved. Schwegler et al. (2005) investigated the potential use of a fusion protein consisting of an anti-Id antibody mimicking CEA and murine GM-CSF as a vaccine to overcome the antigen tolerance in CEA transgenic mice. The data obtained from their study suggest that, mice immunized with the single-chain variable fragment of anti-Id mAb 6G6.C4 (scFv6G6.C4) fused with GM-CSF (scFv6G6.C4/GM-CSF) developed significantly enhanced anti-CEA antibody responses to both scFv6G6.C4 and CEA compared with mice immunized with scFv6G6.C4 alone. CEA-specific antibodies developed in mice immunized with fusion protein could bind with CEA-expressing tumor cells *in vitro* and had no cross-reactivity to CEA-related antigens. Therefore, the fusion of growth factors like GM-CSF could be used to improve the vaccine potential of an anti-Id mAb. However, the tumor therapeutic efficacy of this vaccine approach was not evaluated in this study.

The authors have developed a murine anti-Id mAb designated 3H1 (CeaVac, NIH/Titan Pharmaceuticals), which mimics a specific epitope of CEA (Bhattacharya-Chatterjee et al., 1990). The authors' initial studies in C57BL/6 naive mice suggested that 3H1 could be used as a surrogate antigen for CEA and induced high-titer anti-CEA antibody as well as CD4⁺ T-helper (Th1) responses, which protected mice from tumor growth after challenge with lethal doses of syngeneic murine colon carcinoma cells, MC-38-expressing human CEA (Pervin et al., 1997; Baral et al., 2003; Saha et al., 2003). 3H1 also showed

promise as a potential vaccine candidate in the phase I/II clinical trials of colon cancer patients (reviewed in Bhattacharya-Chatterjee et al., 2002). One area of interest has been the development of new immune adjuvants that could improve the potency of 3H1 as a tumor vaccine and the availability of mouse models expressing human CEA allowed the authors to explore this possibility. The CEA transgenic mice developed by Clarke et al. (1998) were used in the authors' studies and these mice express CEA in a tissue-specific manner as observed in humans. Because oligodeoxynucleotides (ODN) containing CpG motifs have been extensively studied for their ability to stimulate activation of the innate and adaptive immune responses (Krieg, 2002) and in the treatment of cancer (Davila et al., 2003; Sandler et al., 2003; van Ojik et al., 2003), an interest has been shown in a select CpG ODN 1826 as a vaccine adjuvant. The vaccine potential of 3H1 was also assessed in the presence of another widely used adjuvant, QS-21. 3H1 coupled to keyhole limpet hemocyanin (KLH) and mixed with Freund's adjuvant was used as a gold standard in this system. 3H1 vaccination with different adjuvants induced both humoral and cellular anti-CEA immunity in CEA transgenic mice. The immune sera developed in mice could lyse CEA-transfected murine colon carcinoma cells, C15 effectively in an antibody-dependent cellular cytotoxicity (ADCC) assay, and the anti-CEA antibody responses were somewhat comparable in each adjuvant-treated group of mice, whereas cellular immune responses were significantly greater when CpG was used as an adjuvant. 3H1-CpG-immunized mice splenocytes showed an increased proliferative CD4⁺ Th1-type T cell response and secreted elevated levels of Th1 cytokines like IL-2 and IFN- γ . This vaccine also induced MHC class I antigen-restricted CD8⁺ T cell responses. In a solid tumor model, C15 tumor growth was significantly inhibited by 3H1 vaccinations. In 3H1-CpG-vaccinated mice, the duration of survival was, however, longer compared to the 3H1-QS21-vaccinated mice. These findings suggest that 3H1-CpG vaccinations can break peripheral tolerance to CEA and induce protective antitumor immunity in CEA transgenic mice (Saha et al., 2006a).

A prerequisite for successful vaccination is the establishment of a long-lasting protective immune response, and in this regard transport of antigen by DCs is probably of key importance to initiate primary immune responses,

thus permitting establishment of immunological memory (Banchereau et al., 2000). Because DCs are attractive vehicles for cancer immunotherapy, the efficacy of bone marrow–derived DCs pulsed with 3H1 as a tumor vaccine has been evaluated. Immunization of CEA transgenic mice with DCs loaded with 3H1 or CEA could reverse CEA unresponsiveness and result in the induction of CEA-specific immune responses and the rejection of C15 tumor growth. However, tumor-protective immunity was more effective in 3H1-pulsed DCs immunized mice as nearly 100% of experimental mice were protected from tumor growth, whereas only 40% of mice were protected from tumor growth when immunized with CEA-pulsed DCs. The tumor-protective immunity was dependent on the activation of both CD4⁺ T cells and CD8⁺ CTLs in an antigen-specific manner and the induction of humoral immune responses were also observed (Saha et al., 2004). The analysis of cellular immune mechanisms involved in tumor rejection of 3H1-pulsed DCs immunized mice suggested that, CD8⁺ CTLs involved in lysis of C15 tumor cells, produced IFN- γ and TNF- α , and expressed Fas ligand (FasL) and TNF-related apoptosis-inducing ligand (TRAIL) in response to C15 cells. CTLs used perforin-, FasL-, and TRAIL-mediated death pathways to lyse C15 cells, although perforin-mediated killing was the predominant lytic mechanism *in vitro*. The cytokines IFN- γ and TNF- α synergistically enhanced surface expression of Fas, TRAIL receptor, MHC class I and class II on C15 tumor cells that increased the sensitivity of tumor cells to CTL lysis and the CTL activity was directed against an epitope defined by the idiopeptide LCD-2, derived from 3H1. Also, analysis of immunized mice splenocytes that had rejected C15 tumor growth revealed up-regulated surface expression of memory phenotype Ly-6C and CD44 on both CD4⁺ and CD8⁺ T cells. Furthermore, mice that had rejected C15 tumor growth, developed tumor-specific immunological memory that helped those mice to survive a second challenge with C15 tumor cells (Saha et al., 2006b). This study was probably the first report documenting the details of the mechanisms of T cell–mediated immunity invoked by an anti-Id vaccine in a clinically relevant animal model.

CTLs are an important effector arm in the antitumor immune response, and the induction of potent CTL responses has been one of the major goals in developing immunotherapeutic strategies for cancer (Melief et al., 2000). Ac-

cumulating evidence, however, suggests that CD4⁺ T cell response also plays a key role in tumor immunity (Pardoll and Topalian, 1998; Toes et al., 1999). CD4⁺ T cells provide important functions for the induction, expansion, and persistence of CD8⁺ CTLs (Kalams and Walker, 1998). Therefore, an optimal antitumor immune response may require the concomitant activations of both CD4⁺ and CD8⁺ T cells. It is the authors' hypothesis that the combination of vaccines that will elicit both tumor-specific CTL and T helper responses will further augment antitumor immune responses. To address this question, a study was performed where the ability of DC-based vaccine consisting of CTL peptide of CEA in combination with 3H1 were used as a potential vaccine candidate to induce antitumor immunity in a clinically relevant mouse model that express both CEA and HLA-A2. To this end, two different HLA-A2-restricted epitopes were selected: CEA₆₉₁₋₆₉₉ (Kawashima et al., 1998) and CEA₆₀₅₋₆₁₃ (designated CAP-1; Tsang et al., 1995). It has been found that, in a therapeutic setting with 7-day-old established MC-38-CEA-A2Kb tumors, mice immunized with agonist peptide for CEA₆₉₁ (YMIGMLVGV) or mice immunized with agonist peptide for CAP-1 (YLSGADLNL, CAP1-6D) along with 3H1 induced significant increase in survival with complete tumor eradication in 50% and 67.5% of the mice, respectively. Tumor-free mice were immune to tumor re-challenge, and adoptive transfer experiments also suggested the induction of long-lived memory response (Saha et al., 2007). These results support the hypothesis that vaccines that activate both CTLs and T helper lymphocytes should be more effective for clinical use.

CEA-BASED VACCINES IN SPONTANEOUS INTESTINAL TUMOR MOUSE MODEL

The use of transplantable CEA-expressing tumor cells in CEA transgenic mice studies presents some important limitations, including the retroviral insertion of CEA without associated regulatory elements into cells that normally do not express the gene and the growth of those tumor cells at ectopic sites (subcutaneously, lung, liver) not authentic to colorectal cancer. The rapid growth of the transplanted tumors fails to mimic the growth characteristics of carcinomas and requires a shortened interval between cancer vaccine administrations, which probably does not permit optimal host immunity. A better development

of the CEA transgenic mouse model would be to induce neoplastic changes within both CEA-positive and CEA-negative tissues, and test the ability of therapeutic strategies to treat such tumors in immunocompetent animals against the background of “normal” CEA expression. This approach would provide a more realistic indication of their potential to treat gastrointestinal cancer in humans. Several natural mutant mice have been identified that are prone to tumors in specific organs (Mukherjee, et al., 2000; Johnson et al., 2001). The multiple intestinal neoplasia (MIN) mouse has a nonsense mutation in the adenomatous polyposis coli (APC) gene that results in the formation of multiple intestinal adenomas from birth through the first 3 months of development (Moser et al., 1990). The CEA transgenic mouse has been crossed with the MIN mouse to generate a line that spontaneously develops human CEA-expressing adenomas in the intestine (Thompson et al., 1997). So far two studies have been reported using this CEA/APC^{MIN} mouse model to evaluate CEA-based vaccine efficacy.

In CEA-based DNA vaccines, the use of recombinant vaccinia and recombinant fowlpox vectors expressing B7.1, ICAM-1, and LFA-3 (TRICOM) combined with GM-CSF resulted in the induction of CEA-specific humoral and cellular immune responses that significantly suppressed intestinal tumor load and improved long-term survival of CEA/APC^{MIN} mice. The vaccination was initiated at ~30 days of age with monthly booster vaccinations given for the entire length of the study. This aggressive vaccination protocol was able to reduce the number of intestinal tumors without any evidence of autoimmunity directed against normal tissues expressing CEA (Greiner et al., 2002). The same research group designed another study to investigate whether cyclooxygenase-2 (COX-2) and CEA could be simultaneously targeted in a combined chemoimmuno-based approach for cancer prevention and/or therapy (Zeytin et al., 2004). Beginning at 30 days of age, the use of a diversified prime/boost recombinant CEA-TRICOM-poxvirus-based vaccine or the administration of dietary celecoxib (Celebrex), a potent anti-inflammatory drug, which targets COX-2 enzyme, reduced the number of intestinal neoplasms by 54% and 65%, respectively, in CEA/APC^{MIN} mice. The combination of CEA-based vaccine and celecoxib-supplemented diet reduced the tumor burden by 95% and significantly improved overall long-term survival. Although, the combined

treatment with the CEA-based vaccine and dietary celecoxib resulted in enhanced induction of CEA-specific humoral and cellular immune responses, the specific mechanisms involved in antitumor therapy require further studies.

CONCLUSIONS

Reliable animal models can provide useful preclinical information about novel immunotherapies and cancer vaccines as well as for defining tumor immunology paradigms. Transgenic mouse models have allowed tumor immunologists to study the effects of different immunotherapy approaches on cancer and the results obtained using these mice are currently being further evaluated in clinical settings. Although CEA transgenic mouse models are extremely useful for the study of therapeutic efficacy versus toxic effects, crossing these mice with HLA or effector molecule transgenic mice will lead to the generation of mouse models even more relevant to the treatment of human disease.

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