Generation of Human Cytotoxic T Cells Specific for Human Carcinoembryonic Antigen Epitopes From Patients Immunized With Recombinant Vaccinia-CEA Vaccine

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Background: **The human carcinoembryonic antigen (CEA), which is expressed in several cancer types, is a potential target for specific immunotherapy using recombinant vaccines. Previous studies have shown that when the CEA gene is placed into vaccinia virus, the recombinant vaccine (rV-CEA) can elicit T-cell responses in both rodents and nonhuman primates.** ***Purpose:*** **Our objective was to determine if rV-CEA could elicit CEA-specific T-cell responses in humans with appropriate human leukocyte antigen (HLA) motifs.** ***Methods:*** **Peripheral blood lymphocytes (PBLs) obtained from patients with metastatic carcinoma, both before and after vaccination with rV-CEA, were analyzed for T-cell response to specific 9- to 11-mer CEA peptides selected to conform to human HLA class I-A2 motifs.** ***Results:*** **While little or no T-cell growth was seen from preimmunization PBLs of patients pulsed with CEA peptides and interleukin 2 (IL-2), T-cell lines were obtained from PBLs of patients after vaccination with one to three cycles of stimulation. Cytolytic T-cell lines from three HLA-A2 patients were established with a 9-amino acid peptide (CAP-1), and the $CD8^+/CD4^+$ double-positive T-cell line (V24T) was chosen for detailed analysis. When autologous Epstein-Barr virus (EBV)-transformed B cells were either incubated with CAP-1 peptide or transduced with the CEA gene using a retroviral vector, they were lysed by the V24T cell line, but allogeneic non-A2 EBV-transformed B cells were not. The SW403 human colon carcinoma cell line, which is CEA positive and HLA-A2 positive, was also lysed by the V24T cell line, while two non–HLA-A2 CEA-positive colon carcinoma cell lines were not. To further confirm the class I HLA-A2 restricted nature of the V24T cytotoxicity, the non–HLA-A2 SW837 CEA-expressing colon carcinoma cell line was infected with a recombinant vaccinia virus expressing the HLA class I-A2 gene, and it became susceptible to V24T lysis. Cells infected with vector alone were not lysed.** ***Conclusions:*** **This study demonstrates for the first time (*a*) the ability to generate a human cytolytic T-cell response to specific epitopes of CEA, (*b*) the class I HLA-A2 restricted nature of the T-cell mediated lysis, and (*c*) the ability of human tumor cells to endogenously process CEA to present a specific CEA peptide in the context of major histocompatibility complex for T-cell–mediated lysis.** ***Implications:*** **These findings have implications in the development of specific second-generation cancer immunotherapy protocols. [J Natl Cancer Inst 87:982-990, 1995]**

The identification and selection of antigens and specific epitopes as targets for active immunotherapy approaches to human cancer are now in a dynamic phase. Specific peptides that bind human major histocompatibility complex (MHC) molecules have now been identified for melanoma-associated antigens (*1-4*). The identification of human carcinoma-associated antigens and epitopes that can be recognized by human T cells is also currently under active investigation. Molecules, such as prostate specific antigen (PSA) (*5,6*), c-erbB/2 (*7*), MUC-1 (*8*), point mutated ras (*9-11*), point mutated p53 (*12*), and carcinoembryonic antigen (CEA) (*13-15*) are among such candidates.

In humans, CEA is extensively expressed on the vast majority of colorectal, gastric, and pancreatic carcinomas as well as approximately 50% of breast cancers and 70% of non–small-cell lung cancers (*16*). CEA is also expressed, to some extent, on normal colon epithelium and in some fetal tissue (*16*). The CEA gene has been sequenced and shown to be part of the human immunoglobulin gene superfamily (*16,17*) and, thus, shares some homology with other molecules found on normal human tissues. At the amino acid level, CEA shares approximately 70% homology with nonspecific cross-reacting antigen (NCA), which is found on normal granulocytes (*16*).

The immunogenicity of CEA in humans is, at best, controversial. Several studies (*18,19*) claim antibodies to CEA in patients, while other investigators report these observations are artifacts (*20-22*). No reports of the presence or absence of human T-cell responses to CEA exist.

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One strategy that is being pursued to determine if T-cell responses to CEA can be induced in carcinoma patients is to place the CEA gene into vaccinia virus. Vaccinia was chosen as a vector for several reasons. Among these are (*a*) its wide use in humans in the eradication of smallpox; (*b*) its ability to infect a wide range of cells, including professional antigen-presenting cells (APCs), and express the product of an inserted gene such that it has the potential to be processed in the context of class I and/or class II MHC molecules; and (*c*) that animal model studies have shown that the use of a recombinant human CEA vaccinia virus (designated rV-CEA) is superior to the use of soluble CEA in the induction of antitumor effects on established CEA-expressing tumors (*13*). These findings correlated with the appearance of CEA-specific cytotoxic T-lymphocytes (CTLs) in rV-CEA–inoculated animals (*13*). rV-CEA also has been administered to rhesus monkeys and has been shown to induce CEA-specific T-cell responses with no toxicity (*14*).

It is important to emphasize, however, that experimental model results should have extremely limited extrapolation to potential human immune T-cell responses. Human CEA is a foreign gene in both mice and nonhuman primates. However, the more important point to consider is whether human APCs, including tumor cells, will process CEA in such a manner as to present specific CEA peptides in the context of human MHC for human T-cell recognition. Since mouse and nonhuman primate MHC-binding motifs are different from human motifs, studies in animal models cannot answer the question of T-cell immunogenicity in humans. Even the use of CEA-transgenic mice could not answer these questions, because they would possess murine MHC motifs. Thus, while animal model studies were conducted to demonstrate that rV-CEA can infect mammalian cells in vivo to such a level as to induce immune responses and to demonstrate the lack of toxicity, only clinical trials can adequately answer the question of the potential ability of rV-CEA to induce CEA-specific human T-cell responses.

A phase I clinical trial approved by the National Cancer Institute (NCI) Institutional Review Board and conducted by the NCI-Navy Oncology Branch involving the use of rV-CEA in 26 patients with metastatic carcinoma (gastrointestinal, lung, and breast) has recently been completed (*23*). No toxicity was observed other than that usually seen with the smallpox vaccine. A maximum tolerated dose was not achieved, even in the group that received rV-CEA injections of 10^7 plaque forming units (pfu) once a month for 3 months. While T-cell responses to vaccinia virus were observed (*23*), no primary T-cell lymphoproliferative response was observed when soluble CEA protein was presented to peripheral blood lymphocytes (PBLs) obtained prior to or after rV-CEA vaccination.

In an effort to further analyze CEA-specific T-cell responses as a result of rV-CEA vaccination, peptides reflecting potential human class I T-cell epitopes were selected and used with interleukin 2 (IL-2) to stimulate PBLs of patients before and after vaccination with rV-CEA. What has emerged is the first evidence for the induction of CEA-specific CTL responses in patients after rV-CEA vaccination.

Materials and Methods

rV-CEA

A recombinant vaccinia virus expressing CEA (rV-CEA) was generated by Therion Biologics Corporation (Cambridge, MA) using the methods described (*24*). The CEA gene was isolated as a complementary DNA (cDNA) clone from a human colon carcinoma cell cDNA library. The CEA cDNA was inserted, under the control of the vaccinia 40K promoter (*24*), into the *Hind* III M region of the genome of the attenuated strain of vaccinia virus (Wyeth strain).

Cell Cultures

Colorectal carcinoma cell lines SW403 (human leukocyte antigen [HLA]-A2 and HLA-A3), HT-29 (HLA-A1 and HLA-A9), SW837 (HLA-A19, -), and SW1417 (HLA-A3, -) were purchased from American Type Culture Collection (Rockville, MD). The cultures were mycoplasma free and were maintained in complete medium (Dulbecco's modified Eagle medium; Life Technologies, Inc. [GIBCO BRL], Grand Island, NY) supplemented with 10% fetal bovine serum (FBS), 2 m*M* glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin (Life Technologies, Inc.). The T2 cell line (transport deletion mutant) (*25*) was provided by Dr. Peter Cresswell (Yale University School of Medicine, New Haven, CT) and was maintained in Iscove's modified Dulbecco's medium (IMDM) containing 10% FBS.

Epstein-Barr virus (EBV)-transformed B cell lines designated as B-Vac24 and B-Vac01, and the B-Vac24 transfected with a retroviral vector containing the CEA gene [designated as B-Vac24(CEA)] were maintained in RPMI-1640 medium supplemented with 10% pooled human AB serum (Pel Freeze Clinical System, Brown Deer, WI), 2 m*M* glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin (Life Technologies, Inc.).

Peptide Synthesis

The peptide sequence of CEA was scanned for matches to the consensus motifs for HLA-A2 and HLA-A3 binding peptides. HLA-A2 and HLA-A3 alleles were chosen, since they are the most commonly expressed class I alleles. Peptides (9-, 10- and 11-mers) were selected for synthesis if they (*a*) conformed to the respective consensus motifs and (*b*) diverged sufficiently from NCA and human biliary glycoprotein (BGP), so that a response to these antigens would not be anticipated. A peptide that corresponded to the CAP-1 peptide (*see* below) after optimal alignment with NCA and CEA was also synthesized and designated NCA-1. Syntheses were performed on a peptide synthesizer (model 432A; Applied Biosystems, Foster City, CA), and products were dissolved in aqueous solution, sterile filtered, and frozen at –70 °C at a concentration of 2 mg/mL. The purity of the peptides was greater than 90% as analyzed by high-performance liquid chromatography (HPLC). The CEA peptides are listed in Table 1.

Introduction of the CEA cDNA in EBV-Immortalized B-Cell Lines

Since tumors from rV-CEA vaccinated patients were not available, EBV-transformed B cells from these patients were used as autologous targets for T cells. B-cell lines were generated by a standard method (*26*) using B95-8 marmoset cell line supernatant containing EBV. Human pooled AB serum was used in all cell cultures in this study. EBV-immortalized B-cell lines were transduced with a retroviral expression construct of CEA (*27*). Transduction was performed by cocultivation of EBV-immortalized B cells with productively transduced amphotropic retroviral packaging cell line PA317-CEA as described by Tsang et al. (*28*). EBV-immortalized B-cell transductants were selected in medium containing G418 (geneticin) at an active concentration of 0.7 mg/mL. The purpose of the generation of immortalized cells was to have a continuous supply of targets to analyze cytotoxic T-cell responses.

Generation of T-Cell Lines

Peripheral blood mononuclear cells (PBMCs) were obtained from heparinized blood from patients with metastatic carcinoma who were enrolled in a phase I trial employing rV-CEA (*13,14*). All experiments involving patient materials were conducted according to NIH guidelines and written, informed consent was obtained from all patients. PBMCs were obtained prior to and after administration of injections of rV-CEA given once a month for 3 months at 2 ×

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Table 1. Binding of CEA peptides to the HLA class I-A2 molecule

Peptide	Amino acid position in CEA	Sequence	Predicted binding to HLA class I-A2*	T2 binding assay†	
				Experiment 1	Experiment 2
CAP-1	571-579	YLSGANLNL	P	561	806
CAP-2	555-564	VLYGPDTPII	P	515	796
CAP-3	87-96	TLHVIKSDLV	P	480	ND
CAP-4	1-11	KLTIESTPFNV	P	441	ND
CAP-5	345-354	TLLSVTRNDV	P	405	ND
CAP-6	19-28	LLVHNLPQHL	P	381	ND
CAP-7	27-35	HLFGYSWYK	N	326	ND
CAP-8	523-532	TLFNVTRNDA	N	260	ND
CAP-9	137-146	TQDATYLWWV	N	204	ND
CAP-10	102-110	GQFRVYPEL	N	201	ND
NCA-1	571-579	YRPGENLNL	N	252	225
Positive control	—	ALAAAAAAV	P	632	—
No peptide	—	—	—	280	300

*Predicted binding on the basis of published motifs (*31*); P = positive; N = negative.

†Reactivity of T2 cells with anti–HLA-A2 MAb after the cells were incubated with CEA peptide. Peptides were used in a concentration of 50 μg/mL/10^6 cells. The results are expressed in relative fluorescence values (350 was arbitrarily chosen as a cutoff value for positive). Positive control 9-mer is an A2-binding motif.

10^5 pfu (patient Vac7), 2×10^6 pfu (patient Vac6), and 10^7 pfu (patients Vac24, Vac23, and Vac32) per injection. PBMCs from patients were separated using lymphocyte separation medium gradient (Organon Teknika, Durham, NC) as previously described (*29*). Washed PBMCs were resuspended in complete medium: RPMI-1640 (Life Technologies, Inc.) supplemented with 10% pooled human AB serum (Pel Freeze Clinical System), 2 m*M* glutamine, 100 U/mL penicillin, and 100 μg/mL of streptomycin (Life Technologies, Inc.). Cells (2×10^5) in complete medium in a volume of 100 μL were added into each well of a 96-well flat-bottom assay plate (Corning Costar Corp., Cambridge, MA). Peptides were added to cultures at a final concentration of 50 μg/mL. Cultures were incubated for 5 days at 37 °C in a humidified atmosphere containing 5% CO_2. After removal of the peptide-containing medium, the cultures were then provided with human IL-2 (provided by the NCI Surgery Branch) (10 U/mL) for 11 days, with IL-2–containing medium being replenished every 3 days. The incubation time of 5 days with peptide plus 11 days with IL-2 constitutes one cycle. Primary cultures were restimulated with the same peptide (50 μg/mL) on day 16 to begin the next cycle. Irradiated (4000 rad) autologous peripheral blood mononuclear cells (5×10^5) were added in a volume of 50 μL in complete medium as APCs. T-cell lines derived from patients Vac24, Vac6, Vac7, etc. were given the designations V24T, V6T, V7T, etc., respectively.

Cytotoxicity Assays

Various target cells were labeled with 50 μCi of ^{111}In-oxyquinoline (Medi-Physics Inc., Arlington, IL) for 15 minutes at room temperature. Target cells (0.5×10^4) in 100 μL of complete medium (see below) were added to each of 96 wells in U-bottom assay plates (Corning Costar Corp.). The labeled targets were incubated with peptides at a final concentration of 50 μg/mL for 60 minutes at 37 °C in CO_2 before adding effector cells. Effector cells were suspended in 100 μL of complete medium supplemented with 10% pooled human AB serum and added to target cells; the plates were then incubated at 37 °C in 5% CO_2 for 12 or 18 hours. Supernatant was harvested for gamma counting with the use of harvester frames (Skatron, Inc., Sterling, VA). Determinations were carried out in triplicate and standard deviations were calculated. All experiments were carried out three times, except for data in Table 7, which was done twice. Specific lysis was calculated with the use of the following formula:

$$\% \text{ lysis} = \frac{\text{observed release (cpm)} - \text{spontaneous release (cpm)}}{\text{total release (cpm)} - \text{spontaneous release (cpm)}} \times 100.$$

Spontaneous release was determined from wells to which 100 μL of complete medium was added. Total releasable radioactivity was obtained after treatment of targets with 2.5% Triton X-100.

Detection of Tumor Necrosis Factor-α

Supernatants of T cells exposed for 3 days to peptides and APC in IL-2–free medium, at a responder to stimulator ratio of 4:1 (4×10^6:1×10^6 cells/mL), were screened for the secretion of tumor necrosis factor-α (TNF-α), using an enzyme-linked immunosorbent assay kit (Genzyme Corp., Cambridge, MA). The results were expressed in pg/mL.

Flow Cytometry

The procedure for single-color flow cytometric analysis has been previously described (*30*). Briefly, 1×10^6 cells were washed three times with cold Ca^{2+}- and Mg^{2+}-free Dulbecco's phosphate-buffered saline (DPBS) and then stained for 1 hour with 1 μg of monoclonal antibody (MAb) against CD3 (Becton Dickinson, San Jose, CA), CD4 (Becton Dickinson), CD8 (Becton Dickinson), HLA class I (W6/32) (Sera-Lab, Sussex, England), HLA class II (HLA-DR) (Becton Dickinson), and MOPC-21 (Cappel/Organon Teknika Corp., West Chester, PA) in a volume of 100 μL of PBS containing 1% bovine serum albumin. Anti-CEA MAb COL-1 was used as 100 μL of culture supernatant. The cells were then washed three times with cold DPBS and incubated for an additional hour in the presence of 1:100 dilution (volume of 100 μL PBS containing 1% bovine serum albumin) of fluorescein-conjugated goat anti-mouse immunoglobulin (Ig) (Kirkegaard & Perry Labs., Gaithersburg, MD). The cells were again washed three times with DPBS and resuspended in DPBS at a concentration of 1×10^6 cells/mL. The cells were immediately analyzed using a Becton Dickinson FACScan equipped with a blue laser with an excitation of 15 nW at 488 nm. Data were gathered from 10 000 live cells, stored and used to generate results.

The procedure for dual-color flow cytometric analysis was similar to that for single-color analysis, with the following exceptions. The antibodies used were anti-CD4 fluorescein conjugate, anti-CD8 phycoerythrin conjugate, anti-IgG_1 fluorescein conjugate, and anti-IgG_{2a} phycoerythrin conjugate (Becton Dickinson). Staining was done simultaneously for 1 hour after which cells were washed three times, resuspended as above, and immediately analyzed using a Becton Dickinson FACSort equipped with a blue laser with an excitation of 15 nW at 488 nm equipped with the Lysis II program.

Binding of CEA peptides to the HLA-A2 molecule was analyzed by the up-regulation of HLA-A2 expression on T2 cells (*25*) as demonstrated by flow cytometry. The T2 cell peptide binding assay has been reported previously (*31*). Briefly, aliquots of $0.5\text{-}1 \times 10^6$ T2 cells in serum-free IMDM were incubated with peptides at a concentration of 50 μg/mL in 24-well culture plates at 37 °C in 5% CO_2 overnight. Flow cytometry for peptide binding was carried out using T2 cells and single-color analysis. After cells were washed three times in DPBS as above, they were incubated for 1 hour with HLA-A2 specific antibody A2,28 (#189HA-1; One Lambda, Inc., Canoga Park, CA), using 10 μL of a 1× working dilution/10^6 cells. MOPC-104E (Cappel/Organon Teknika Corp., West Chester, PA) was used as isotype control. The cells were then washed three times and incubated with a 1:100 dilution of phycoprobe PE (phycoerythrin) labeled anti-mouse IgM (Biomeda Corp., Foster City, CA). Analysis was carried out using the FACScan as described above. Cells were maintained on ice during all cell preparation and staining unless otherwise stated above.

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HLA Typing

The HLA phenotyping of patients was performed by the Tissue Typing QC Laboratory, Naval Medical Research Institute, Bethesda, MD, using a standard antibody-dependent microcytotoxicity assay and a defined panel of anti-HLA antisera. The HLA phenotypes were as follows: patient Vac24 (HLA-A2, 24; B 44, 51; DR 4, 11; DQ 3, 7; DR w52, 53); patient Vac01 (HLA-A28, 31; B14, 35; DR1, 4; DQ1, 3; DRw53); patient Vac23 (HLA-A1, 26; B8, 60; CW3, 7; DR0103, 15; DQ5, 6); patient Vac32 (HLA-A3, 68; B7, 51; CW7; DR4, 15; DQ1, 8; DRw53); patient Vac6 (HLA-A2, 24; B13, 51; CW6; DR7, 8; DQ4; DR53); and patient Vac7 (HLA-A2; B7; CW7; DR15, 17; DQ1, 2; DR52). From patient Vac01, only B cells were used.

Vaccinia Virus Infection of Colorectal Carcinoma Cells

cDNA for the HLA-A2.1 gene in the vaccinia virus vector was provided by the Surgery Branch, NCI, National Institutes of Health. These genes were inserted into the TK gene in plasmid pSCII, allowing homologous recombination to occur with the viral TK gene (*32*). Target cells at a concentration of 1×10^7/mL in complete RPMI-1640 medium supplemented with 0.1% bovine serum albumin were incubated with an equal volume of vaccinia virus (10^8 pfu/mL) in the same medium at 37 °C for 1.5 hours. The cells were then adjusted to a concentration of 5×10^5/mL in complete medium and incubated for 3 hours at 37 °C.

Statistical Analysis

Statistical analysis of differences between means was done by a two-tailed paired *t* test.

Results

Identification of Potential CEA-Specific T-Cell Epitopes

Since the entire amino acid sequence of human CEA is known and human HLA class I-A2 consensus motifs have been described (*33,34*), studies were undertaken to identify a series of peptides that would potentially bind class I-A2 molecules. A2 was chosen, since it is the most common HLA class I molecule, being represented in approximately 50% of North American caucasians and 34% of African-Americans (*35*). The peptide sequence of CEA was thus examined for matches to the consensus motifs for HLA-A2 binding peptides. Peptides were only selected, moreover, if their sequence diverged sufficiently from the CEA-related NCA and BGP sequences. The amino acid sequence of human CEA (GeneBank Accession #M17303) was scanned using a predictive algorithm (*36*) that combines a search for anchor residues with numerical assignments to all residues at all positions. Ten peptides were synthesized using this algorithm, ranging in length from 9 to 11 amino acids. Six of these peptides also contained the HLA-A2 binding motif of leucine or isoleucine at position 2 and valine or leucine at the C terminal. Another peptide (CAP-7) also possessed the motif for binding to HLA-A3 (*37*). All peptides were selected to have minimal homology to the parallel regions of NCA and BGP after optimal alignment of the latter sequences with CEA. The 9-mer, 10-mer, or 11-mer peptides that met these criteria were selected for synthesis and purification; they were designated CAP (carcinoembryonic antigen peptide)-1 through 10. Their amino acid sequence and position in the CEA molecule are given in Table 1. The positive (P) or negative (N) designation (Table 1) relates to the predicted binding to HLA-A2.

The T2 cell-binding assay has been used to predict human HLA-A2 consensus motifs (*31*). In this assay, the binding of an appropriate peptide results in the up-regulation of surface HLA-A2 on the T2 cells, which can be quantified via FACScan using an anti–HLA-A2 antibody. As seen in Table 1, six of the CEA peptides (CAP-1 through CAP-6) scored positive for T2 binding (the peptides were designated CAP-1 through CAP-10 retrospectively on the basis of their quantitative binding to T2). In general, the peptides with the HLA-A2 consensus motif were better binders than those lacking the motif. The order of T2 cell-peptide binding did not always correspond to the predictive algorithm (*36*). Since peptide 571-579 (designated CAP-1) demonstrated the highest level of T2 binding, the peptide reflecting the NCA analog (the corresponding NCA peptide obtained after optimal alignment of NCA and CEA) was also synthesized and tested; this peptide, designated NCA-1, showed background binding to T2 cells (Table 1). This low level of binding was consistent with the fact that an amino acid substitution in NCA had abolished one of the A2 anchor residues (Arg for Leu at position 2).

Establishment of T-Cell Lines to CEA Peptides

In an attempt to establish T-cell lines from patients who had received the rV-CEA construct, PBLs were obtained from three patients (designated Vac6, Vac7, and Vac24) with the HLA-A2 allele and were alternately pulsed with 50 μg/mL peptide CAP-1 and IL-2 (10 U/mL) as described in the "Materials and Methods" section. In all three cases, T-cell lines could be established that were cytotoxic for T2 cells when pulsed with the CAP-1 peptide. Fig. 1 shows the results of these assays using T-cell lines from patients Vac24 and Vac6. The T-cell line from patient Vac24 was chosen for further study.

PBLs from patient Vac24 (before and after vaccination with three doses of 10^7 pfu rV-CEA at monthly intervals) were placed in 96-well plates and pulsed with the CAP-1 peptide and then IL-2, as described in the "Materials and Methods" section. Each exposure to peptide and IL-2 was considered one cycle of stimulation. As seen in Table 2, one, two, or three cycles of CAP-1 peptide and IL-2 did not result in growth of cells in any of the 96 wells using the preimmunization PBLs. By contrast, after one cycle of stimulation of post-vaccination PBLs from the same patient, 66 of the 96 wells (69%) demonstrated growth of cells, which were maintained through four cycles of stimulation. It is of interest that, after four cycles of stimulation of preimmunization PBLs, two (2%) of 96 wells exhibited cell growth. Thus, one could hypothesize that a minor population of T cells exists in this patient that is capable of recognizing a specific CEA epitope (peptide 571-579) and that these cells were clonally expanded as a result of the rV-CEA administration.

Sufficient PBLs, before and after vaccination with rV-CEA at the 10^7 pfu dose, were also available from two non–HLA-A2 patients: Vac32 (HLA A1,26) and Vac23 (HLA A3,68). Since we had little or no basis for predicting which peptides might bind to these haplotypes, nine of the CEA peptides were used in an attempt to establish T-cell lines. Using peptide CAP-1 with IL-2 as described above, no T-cell lines could be established from preimmunization PBLs from either patient Vac32 or Vac23 (Table 2). However, employing post–rV-CEA immunization PBLs, T-cell lines were established after three cycles of stimulation in 25 (52%) of 48 wells for patient Vac32 and in 21 (44%) of 48 wells for patient Vac23 (Table 2).

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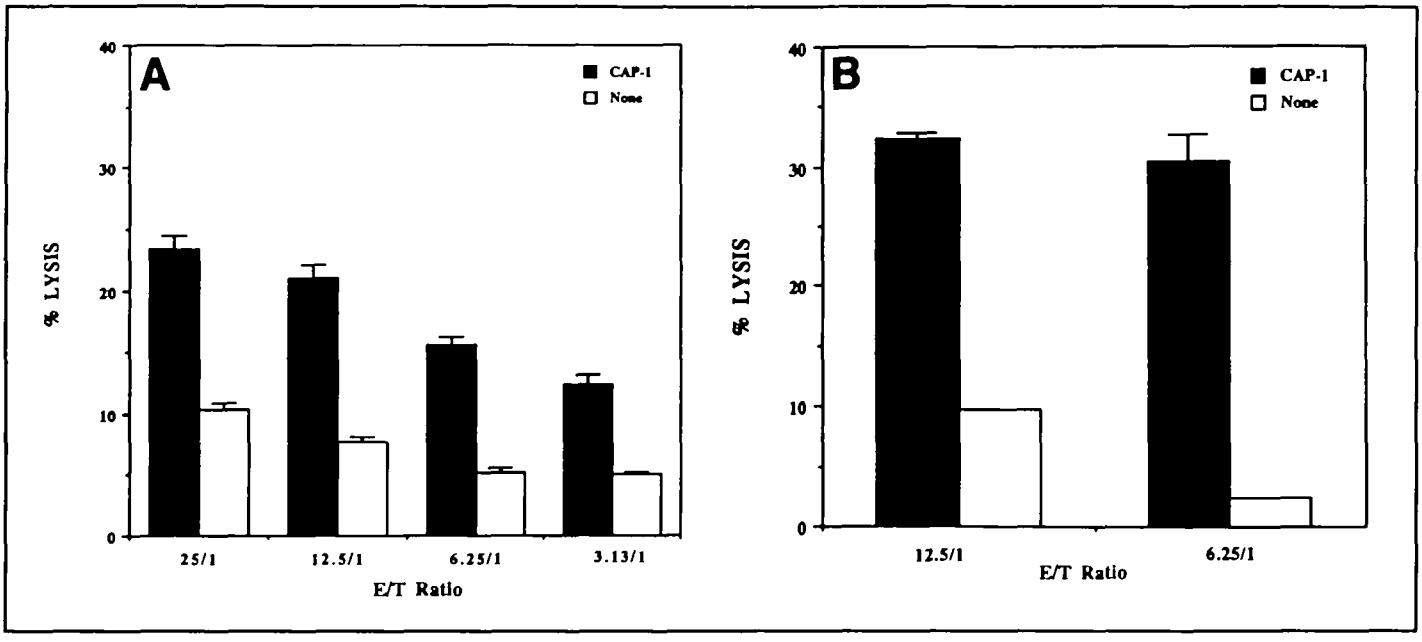

Fig. 1. Cytotoxicity of T-cell lines (designated V24T [Panel A] and V6T [Panel B]) derived from patients immunized with rV-CEA and induced by CEA CAP-1 peptide. CTL activity was determined in an 18-hour ^{111}In release assay using T2 cells as a target incubated with CAP-1 peptide (50 μg/mL). E/T ratio = effector-to-target ratio.

A similar contrast in pre-vaccination versus post-vaccination PBLs from patients Vac32 and Vac23 was seen with a mixture of CEA peptides CAP-4, CAP-6, and CAP-7 (Table 2). Combinations of peptides were used to conserve PBLs. It is of interest that PBL from patient Vac32 (HLA-A3 positive) showed evidence of cell growth in the presence of CAP-7, the peptide that bears the HLA-A3–binding motif. It should be noted that these results suggest that a peptide shown to bind to HLA-A2 can also stimulate T-cell lines after binding to some non-A2 antigens; the possible reasons for this will be discussed in detail below. However, it was decided to first characterize the T-cell responses in patient Vac24 because of the implied relevance of MHC binding and T-cell activation. Nonetheless, it is encouraging that PBLs from five of five patients showed signs of T-cell response to peptide CAP-1 after immunization with rV-CEA.

Flow Cytometry Analysis

Flow cytometric studies were conducted to phenotype the V24T cell line and V6T and V7T cells obtained by pooling respective cells growing in 96-well plates. The results are shown in Table 3. Cells stained double positive for both CD8 and CD4 in V24T and V6T cell lines, while the V7T cell line was CD8 positive.

Cytotoxicity Assays

To determine if the T-cell line from patient Vac24 (designated V24T) could lyse autologous B cells presenting the CAP-1 peptide, B cells from this patient were first transformed with EBV and then pulsed (i.e., incubated) with the CAP-1 peptide. As seen in Table 4, the V24T cells were capable of lysing the

Table 2. Frequency of T-cell growth following an in vitro stimulation with CEA peptides: before and after immunization with rV-CEA

		Frequency							
		Before immunization*				After immunization*			
Patient	Peptide	1	2	3	4	1	2	3	4
Vac24†	CAP-1	0	0	0	2	66	66	66	66
Vac32‡	CAP-4, CAP-6, CAP-7	0	0	4	ND	0	0	30	ND
	CAP-3, CAP-10, CAP-9	0	0	0	ND	0	0	0	ND
	CAP-8, CAP-2	0	0	0	ND	0	0	0	ND
	CAP-1	0	0	0	ND	0	0	25	ND
Vac23‡	CAP-4, CAP-6, CAP-7	0	0	3	ND	0	0	31	ND
	CAP-3, CAP-10, CAP-9	0	0	0	ND	0	0	0	ND
	CAP-8, CAP-2	0	0	0	ND	0	0	0	ND
	CAP-1	0	0	0	ND	0	0	21	ND

*Cycle of stimulation with peptide and IL-2 (*see* "Materials and Methods" section); ND = not done.

†Results are expressed as the number of positive wells per 96 wells. Peripheral blood mononuclear cells were seeded at a concentration of 1×10^5 cells per 200 μL/well.

‡Results are expressed as the number of positive wells per 48 wells. Peripheral blood mononuclear cells were seeded at a concentration of 1×10^6 cells per well in 1 mL.

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Table 3. Flow cytometric analysis of surface markers on T-cell lines*

Surface antigen†	Cell line V24T	V6T	V7T
$CD8^+/CD4^+$	62.4	30.8	Negative
$CD8^+/CD4^-$	35.7	56.0	99.6
$CD4^+/CD8^-$	Negative	12.6	Negative
$CD3^+$	98.5	95.4	99.4

*Negative = less than 5% positive. Results are expressed in percentage of each T-cell line reactive with the MAbs. Routinely, 2%-4% of the cells were stained when treated either with no primary MAb or an isotype-matched control MAb.
†+ = positive; – = negative.

Table 4. Ability of V24T cells (T cells derived from PBLs from rV-CEA–vaccinated patient Vac24) to lyse autologous and allogeneic B cells pulsed with a CEA-specific peptide*

B cells	HLA-A2†	Pulsing peptide	% lysis (± SD)
B-Vac24	Positive	CAP-1	43 (0.29)†
		NCA-1	8 (1.92)
		None	2 (0.37)
B-Vac01	Negative	CAP-1	10 (0.53)
		NCA-1	11 (0.62)†
		None	8 (0.68)

*An 18-hour ^{111}In release assay was performed. Peptides were used in a concentration of 50 μg/mL. Results are expressed as percentage specific lysis at effector-to-target ratios of 25:1.
†Statistically significant ($P<.01$, paired t test). Similar statistically significant lysis was observed at effector-to-target ratios of 12.5:1.

autologous B cells when pulsed with CAP-1, but when an allogeneic (non–HLA-A2) EBV-transformed B cell was pulsed with the same peptide, no lysis was observed. Lysis was observed at effector-to-target cell ratios of 25:1 and 12.5:1. When the NCA-1 peptide, reflecting the analogous region on the NCA molecule was used to pulse B cells of patient Vac24, no lysis was observed with the V24T cells. As shown in Table 1, this lack of lysis was not unexpected, since three of the nine amino acids of NCA-1 differ from those of CAP-1, including an anchor residue.

Studies were then undertaken to determine if the CAP-1 peptide could induce the secretion of TNF-α from the cytolytic V24T cells. Incubation of V24T cells with autologous B cells pulsed with CAP-1 peptide resulted in the production of more than 300 pg/mL of TNF-α, while incubation with control or non-T2 cell-binding peptides CAP-9 and CAP-10 or no peptide showed levels of production below 75 pg/mL.

Cytotoxicity of V24T Cells Against Tumor Cells

While the above studies indicate that autologous B cells can present the CAP-1 peptide to the V24T cells, resulting in lysis of the B cells, they do not indicate that human APCs can endogenously process the entire CEA molecule in a manner so as to bind HLA-A2 molecules for presentation at the cell surface. To help clarify this issue, EBV-transformed B cells of patient Vac24 were transduced with the entire human CEA gene, using a retroviral vector (*see* "Materials and Methods" section). As seen in Table 5, the CEA-transduced cells now express CEA, and the transduction process had no effect on the expression of HLA class I and class II molecules.

Table 5. Flow cytometric analysis of surface antigens of EBV-transformed B cells derived from patient Vac24 before and after transfection with CEA

Antigen	MAb	% positive* B-Vac24†	B-Vac24 (CEA)‡
CEA	COL-1	4.4 (24.7)	42.9 (100.9)
HLA class I	W6/32	100.0 (831.9)	100.0 (519.1)
HLA class II	anti–HLA-DR	99.8 (313.3)	99.3 (221.8)
Control	MOPC-21	2.2 (30.5)	2.0 (24.1)

*Values represent the percentage of each cell type reactive with MAbs listed as analyzed by flow cytometry. Numbers in parentheses are the mean channel fluorescence intensity as determined in relative log units. Routinely, 2%-4% of the cells were stained when treated either with no primary MAb or an isotype-matched control MAb. LS-174T, a colorectal carcinoma cell line, was used as a positive control for CEA expression. The percent positive value for CEA in CEA-expressing LS-174T human colon carcinoma cell line was 59.8 (144.4).
†B-Vac24 are EBV-transformed B cells derived from PBLs of patient Vac24 prior to immunization with rV-CEA.
‡B-Vac24(CEA) are the same as B-Vac24 except they have been transduced with the entire human CEA gene using a retroviral vector as described in the "Materials and Methods" section.

As shown in Table 6, the autologous B cells transduced with the CEA gene can now serve as targets for the V24 CTLs. These results thus demonstrate that a CEA gene product can be endogenously processed by autologous B cells and presented at the cell surface in the context with class I MHC to induce T-cell lysis. The question now remained as to whether human carcinoma cells can act in the same manner as APCs and, thus, serve as potential targets for V24T cells. As seen in Table 6, non-A2 allogeneic carcinoma cells SW1417 and HT-29, which do express substantial CEA, cannot serve as targets, while the allogeneic A2-positive SW403 carcinoma cells expressing CEA are lysed at effector-to-target ratios of 50:1 and 25:1.

Cytotoxicity of V24T Cells Against Vaccinia-CEA–Infected Tumor Cells

To further demonstrate the HLA-A2 restricted nature of the V24T cells in the lysis of human carcinoma cells, the CEA-positive, non–HLA-A2 SW837 human carcinoma cell line was employed. These cells were either uninfected, infected with wild-type vaccinia virus, or infected with a recombinant vaccinia virus containing the HLA-A2 gene. Twelve-hour lysis ex-

Table 6. Cytotoxicity of V24T cell line (derived from patient Vac24 immunized with rV-CEA) on target cells with endogenous CEA expression*

Target	HLA-A2	CEA	% lysis (± SD)
B-Vac24†	Positive	Negative	8.2 (2.1)
B-Vac24 (CEA)†	Positive	Positive	46.1 (11.6)§
SW403‡	Positive	Positive	45.2 (1.5)§
SW1417‡	Negative	Positive	5.2 (0.5)
HT-29‡	Negative	Positive	4.1 (0.6)

*HLA-A2 and CEA expression were tested by flow cytometry using MAbs anti-A2 and COL-1, respectively. An 18-hour ^{111}In release assay was performed. Results are expressed in percent specific lysis at effector-to-target ratio of 50:1 compared with lysis obtained with B-Vac24 cells. Similar statistically significant lysis was seen at effector-to-target ratio of 25:1.
†As described in the legend to Table 4.
‡Human colon carcinoma cell lines expressing CEA.
§Statistically significant lysis ($P<.01$, paired t test).

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periments were carried out to avoid spontaneous lysis due to vaccinia virus. As seen in Table 7, only the carcinoma cells infected with the rV-A2.1 recombinant expressing HLA-A2 were susceptible to lysis with V24T cells. These studies further demonstrate the HLA-A2 restricted nature of the CEA-specific lysis of the V24T cells.

Discussion

These studies demonstrate that one can evoke a cytotoxic T-cell response to a specific epitope of the human CEA molecule by vaccination with rV-CEA. This response appears to be mediated via a class I MHC restricted mechanism. Moreover, the lytic T cells generated against the defined CEA peptide were able to lyse tumor cells endogenously synthesizing the entire CEA antigen. The ability to raise a CTL response versus CEA in humans was clearly not a foregone conclusion prior to these studies; since CEA is expressed in fetal tissue and some normal colonic mucosa, "tolerance" to this molecule was one possible outcome. Moreover, previous studies dealing with the presence of antibodies to CEA were inconclusive and contradictory (*38-41*), and no studies have reported either the presence or the absence of cytolytic T-cell responses to CEA in humans. It was for this reason that the CEA gene was placed into vaccinia virus. A previous study (*13*) in a mouse model demonstrated enhanced CTL responses when employing rV-CEA as an immunogen as opposed to native CEA.

Other data are currently being accumulated on the potential immunogenicity of CEA. While the patients in the study reported here had advanced metastatic disease, a trial using the same rV-CEA construct described here, at the same dose schedule and route of administration, is currently under way in gastrointestinal cancer patients with minimal disease. In those studies, primary lymphoproliferative T-cell responses to CEA have been observed (*42*). In another study (*43*), an anti-idiotype MAb to an anti-CEA MAb has been administered to gastrointestinal cancer patients; in that study, antibodies to CEA and lymphoproliferative responses to CEA were reported. It should also be noted that a pilot phase I study (*44*) was previously carried out in which soluble CEA was administered to patients with advanced gastrointestinal carcinomas. In that study, as well as the other studies cited, no toxicity was observed. The findings of these phase I trials thus indicate that CEA deserves further evaluation as a potential target for immunotherapeutic applications.

Table 7. Demonstration of HLA-A2 involvement in ability of V24T cells to lyse human colon carcinoma cells*

Infection	Expression of MHC class I molecules†		% lysis (± SD)‡
	W6/32 antibody	A2,28 antibody	
None	99.1 (228.23)	4.4 (47.41)	8.2 (1.4)
Vaccinia A2.1	99.3 (261.74)	90.4 (66.70)	31.4 (2.5)§
Vaccinia vector control	99.4 (217.59)	2.9 (17.54)	7.2 (0.6)

*SW837, an HLA-A2–negative and CEA-positive human colorectal carcinoma cell line, was infected with vaccinia A2.1 vector. The surface expression of HLA-A2 molecule after infection was analyzed by flow cytometry using anti–HLA-A2 MAb (A2.28) and anti-HLA class I antibody (W6/32).

†Values represent the percentage of cells reactive with the antibodies listed. Numbers in parentheses are the mean fluorescence intensity as determined in relative log units.

‡Results are expressed in percent specific lysis in a 12-hour ^{111}In release assay at effector-to-target ratio of 50:1.

§Statistically significant lysis ($P<.01$, paired t test).

The finding reported here that the administration of rV-CEA can elicit a T-cell response to a defined 9-mer epitope on CEA leads to potential second-generation protocols. One of the limitations on the use of a vaccinia recombinant vaccine is that it elicits a strong antibody response to the vaccinia virus (*13*). This in turn, prohibits numerous boosts with the recombinant vaccinia, due to the anti-vaccinia antibody inhibition of local virus spread. Indeed, this boost in anti-vaccinia antibody, seen in the phase I trial with rV-CEA, may be a limitation in the sole use of rV-CEA as immunogen.

Now that a specific epitope on the CEA molecule has been identified, subsequent studies in HLA-A2 patients can be designed in which one or two rV-CEA administrations are followed by several administrations of the CAP-1 peptide (amino acid 571-579) in adjuvant or liposomes as boosts. Moreover, protocols involving the use of rV-CEA and recombinant CEA protein, anti-idiotype MAb (as surrogate antigen for CEA), or other viral vectors may be considered. It should be pointed out that the T cells recognizing the CAP-1 peptide do not recognize the corresponding sequence on NCA. In addition, the specificity of V24T was demonstrated by the induction of TNF-α secretion by incubation of V24T with autologous B cells pulsed with CAP-1 but not with CAP-9 or CAP-10 peptides.

An alternative approach would be to expand in vitro the CTL lines derived from PBLs of rV-CEA–immunized HLA-A2 patients by pulsing with the CAP-1 peptide plus IL-2. These antigen-specific CTLs could then be adoptively transferred to the autologous patient. The adoptive transfer of antigen-specific CTL could conceivably be followed by administration of the CAP-1 peptide in adjuvant or a liposomal formulation to further expand the CEA-specific CTL population in vivo. Perhaps fewer cells of this type need to be adoptively transferred than in tumor-infiltrating lymphocyte protocols (*45*), since these antigen specific T cells are expanded and activated by a known 9-mer epitope.

The broad use of peptide CAP-1 as an immunogen is an intriguing issue. We saw evidence of T-cell growth and the establishment of CTL lines from post-immunization PBLs from three of three HLA-A2 individuals. Unexpectedly, we also saw indications of cell growth in post-immunization PBLs from two individuals who were negative for the A2 allele. In both of these cases, however, T-cell growth was not seen until after three cycles of stimulation with peptide and IL-2. If in fact these are MHC-restricted T-cell responses, one must postulate that the peptide designed to bind to HLA-A2 molecules can also bind to other HLA class I or II molecules. In principle, this phenomenon could arise from the following scenarios: (*a*) alternate class I or II molecules share consensus motifs with HLA-A2 or (*b*) the CAP-1 peptide fortuitously contains sequences capable of binding more than one HLA class I or II antigen, i.e., contains multiple consensus motifs. The current literature on MHC restriction offers examples of both scenarios. For example, HLA-

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A3 and HLA-A11 molecules are both known to prefer 9-mer peptides with valine at position 2 and lysine at the C terminal (*46*). An example of the second situation is the specific immunity generated in mice of different genetic backgrounds to a defined viral epitope of lymphocytic choriomeningitis virus (*47*).

Less controversial, but equally intriguing, is the suggestion that T-cell growth was observed in vitro in HLA-A3 PBL (patient Vac32) by stimulation with a peptide that also fits the A3 consensus motif. If this HLA-A3 stimulation turns out to be the case, evidence is provided that rV-CEA can induce MHC-restricted CTL responses in individuals with various haplotypes.

The area of T-cell immunotherapy that is now emerging involves at least two vital parts. The first is the identification of the target antigen/epitope in the context of known MHC molecules in terms of recognition by T cells. The second vital step involves T-cell activation and clonal expansion. Thus, studies are now under way to better activate T cells recognizing specific human epitopes. These studies will involve the use of cytokines and T-cell costimulatory molecules such as B7.1. In a recent study (*48*), we have demonstrated that the murine B7.1 and B7.2 molecules can each be placed into a vaccinia virus vector to enhance the immunogenicity of murine carcinoma cells in an experimental model. Studies are currently under way in an experimental model to coadminister rV-CEA with rV-B7 recombinants to enhance the immunogenicity of CEA; preliminary results appear to be promising.

References

(*1*) van der Bruggen P, Traversari C, Chomez P, et al: A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. Science 254:1643-1647, 1991

(*2*) Hom SS, Topalian SL, Simonis T, et al: Common expression of melanoma tumor-associated antigens recognized by human tumor infiltrating lymphocytes: analysis by human lymphocyte antigen restriction. J Immunother 10:153-164, 1991

(*3*) Cox AL, Skipper J, Chen Y, et al: Identification of a peptide recognized by five melanoma-specific human cytotoxic T cell lines. Science 264:716-719, 1994

(*4*) Darrow TL, Slingluff CL Jr, Seigler HF: The role of HLA Class I antigens in recognition of melanoma cells by tumor-specific cytotoxic T lymphocytes. Evidence for shared tumor antigens. J Immunol 142:3329-3335, 1989

(*5*) Oesterling JE: Prostate specific antigen: a critical assessment of the most useful tumor marker for adenocarcinoma of the prostate. J Urol 145:907-923, 1991

(*6*) Peace DJ, Xue BH, Sosman JA, et al: In vitro immunization of human cytotoxic T lymphocytes specific for peptides derived from prostate specific antigen. Cancer Vaccine Symposium. Cancer Research Institute. Oct. 3-5, 1994 [abstr]

(*7*) Fisk B, Chesak B, Ioannides MG, et al: Sequence motifs of human HER-2 proto-oncogene important for peptide binding to HLA-A2. Int J Oncol 5:51-63, 1994

(*8*) Ioannides CG, Fish B, Jerome KR, et al: Cytotoxic T cells from ovarian malignant tumors can recognize polymorphic epithelial mucin core peptides. J Immunol 151:3693-3703, 1993

(*9*) Tsang KY, Nieroda CA, De Filippi R, et al: Induction of human cytotoxic T-cell lines directed against point-mutated p21 ras-derived synthetic peptides. Vaccine Res 3:183-193, 1994

(*10*) Jung S, Schluesener HJ: Human T lymphocytes recognize a peptide of single point-mutated, oncogenic ras proteins. J Exp Med 173:273-276, 1991

(*11*) Fenton RG, Taub DD, Kwak LW, et al: Cytotoxic T-cell response and in vivo protection against tumor cells harboring activated ras proto-oncogenes [*see* comment citation in Medline]. J Natl Cancer Inst 85:1294-1302, 1993

(*12*) Houbiers JG, Nijman HW, van der Burg SH, et al: In vitro induction of human cytotoxic T lymphocyte responses against peptides of mutant and wild-type p53. Eur J Immunol 23:2072-2077, 1993

(*13*) Kantor J, Irvine K, Abrams S, et al: Antitumor activity and immune responses induced by a recombinant carcinoembryonic antigen-vaccinia virus vaccine [*see* comment citation in Medline]. J Natl Cancer Inst 84:1084-1091, 1992

(*14*) Kantor J, Irvine K, Abrams S, et al: Immunogenicity and safety of a recombinant vaccinia virus vaccine expressing the carcinoembryonic antigen gene in a nonhuman primate. Cancer Res 52:6917-6925, 1992

(*15*) Ras E, VanderBerg SH, Warnaar SO: Identification of potential tumor-specific CTL epitopes of epithelial surface antigen (ESA) and carcinoembryonic antigen (CEA). European Immunology Meeting, Barcelona, June 1994 [abstr]

(*16*) Thompson JA, Grunert F, Zimmermann W: Carcinoembryonic antigen gene family: molecular biology and clinical perspectives. J Clin Lab Anal 5:344-366, 1991

(*17*) Oikawa S, Imajo S, Noguchi T, et al: The carcinoembryonic antigen (CEA) contains multiple immunoglobulin-like domains. Biochem Biophys Res Commun 144:634-642, 1987

(*18*) Staab HJ, Anderer FA, Stumpf E, et al: Are circulating CEA immune complexes a prognostic marker in patients with carcinoma of the gastrointestinal tract? Br J Cancer 42:26-33, 1980

(*19*) Mavligit GM, Stuckey S: Colorectal carcinoma. Evidence for circulating CEA-anti-CEA complexes. Cancer 52:146-149, 1983

(*20*) Collatz E, Von Kleist S, Burtin P: Further investigations of circulating antibodies in colon cancer patients: on the autoantigenicity of the carcinoembryonic antigen. Int J Cancer 8:298-303, 1971

(*21*) Chester KA, Begent RH: Circulating immune complexes (CIC), carcinoembryonic antigen (CEA) and CIC containing CEA as markers for colorectal cancer. Clin Exp Immunol 58:685-693, 1984

(*22*) Ura Y, Ochi Y, Hamazu M, et al: Studies on circulating antibody against carcinoembryonic antigen (CEA) and CEA-like antigen in cancer patients. Cancer Lett 24:283-295, 1985

(*23*) Hamilton JM, Chen AP, Nguyen B, et al: Phase I study of recombinant vacinnia virus (rV) that expresses human carcinoembryonic antigen (CEA) in adult patients with adenocarcinomas. Proc ASCO:abstr 961, 1994

(*24*) Gritz L, Destree A, Cormier N, et al: Generation of hybrid genes and proteins by vaccinia virus-mediated recombination: application to human immunodeficiency virus type I env. J Virol 64:5948-5957, 1990

(*25*) Anderson KS, Alexander J, Wei M, et al: Intracellular transport of class I MHC molecules in antigen processing mutant cell lines. J Immunol 151:3407-3419, 1993

(*26*) Blumberg RS, Panadis T, Byington R, et al: Effects of human immunodeficiency virus on the cellular immune response to Epstein-Barr virus in homosexual men: characterization of the cytotoxic response and lymphokine production. J Infect Dis 155:877-890, 1987

(*27*) Robbins PF, Kantor JA, Salgaller M, et al: Transduction and expression of the human carcinoembryonic antigen gene in a murine colon carcinoma cell line. Cancer Res 51:3657-3662, 1991

(*28*) Tsang KY, Kashmiri SV, De Filippi R, et al: A human T cell line engineered to secrete chimeric monoclonal antibody. J Immunother 13:143-152, 1993

(*29*) Boyum A: A one-stage procedure for isolation of granulocytes and lymphocytes from human blood. General sedimentation properties of white blood cells in a 1g gravity field. Scand J Clin Lab Invest 97(suppl):51-76, 1968

(*30*) Guadagni F, Witt PL, Robbins PF, et al: Regulation of carcinoembryonic antigen expression in different human colorectal tumor cells by interferon-γ. Cancer Res 50:6248-6255, 1990

(*31*) Nijman HW, Houbiers JG, Vierboom MP, et al: Identification of peptide sequences that potentially trigger HLA-A2.1-restricted cytotoxic T lymphocytes. Eur J Immunol 23:1215-1219, 1993

(*32*) O'Neil BH, Kawakami Y, Restifo NP, et al: Detection of shared MHC-restricted human melanoma antigens after vaccinia virus-mediated transduction of genes coding for HLA. J Immunol 151:1410-1418, 1993

(*33*) Falk K, Rotzschke O, Stevanovic S, et al: Allele-specific motifs revealed by sequencing of self-peptides eluted from MHC molecules. Nature 351:290-296, 1991

(*34*) Hunt DF, Henderson RA, Shabanowitz J, et al: Characterization of peptides bound to the class I MHC molecule HLA-A2.1 by mass spectrometry. Science 255:1261-1263, 1992

(*35*) Lee J: The HLA System: A New Approach. New York: Springer-Verlag, 1990, p 154

(*36*) Parker KC, Bednarek MA, Coligan JE: Scheme for ranking potential HLA-A2 binding peptides based on independent binding of individual peptide side-chains. J Immunol 152:163-175, 1994

(*37*) DiBrino M, Parker KC, Shiloach J, et al: Endogenous peptides bound to HLA-A3 possess a specific combination of anchor residues that permit identification of potential antigenic peptides. Proc Natl Acad Sci U S A 90:1508-1512, 1993

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(38) Fuchs C, Krapf F, Kern P, et al: CEA-containing immune complexes in sera of patients with colorectal and breast cancer-analysis of complexed immunoglobulin classes. Cancer Immunol Immunother 26:180-184, 1988
(39) Abeyounis CJ, Wilhelm SA, Rittenhouse KW, et al: Is carcinoembryonic antigen an autoantigen? Transplant Proc 16:489-491, 1984
(40) MacSween JM: The antigenicity of carcinoembryonic antigen in man. Int J Cancer 15:246-252, 1975
(41) Steele G Jr, Lahey S, Rodrick M, et al: Circulating immune complexes in patients with colorectal cancer. Am J Surg 145:549-553, 1983
(42) Conry RM, Saleh MN, Schlom J, et al: Breaking tolerance to carcinoembryonic antigen with a recombinant vaccinia virus vaccine in man. Proc Am Assoc Cancer Res 36:abstr 492, 1995
(43) Foon KA, Chakrabarty M, John W, et al: Active immunity to the carcinoembyonic antigen (CEA) in patients treated with an anti-idiotype monoclonal antibody vaccine. Proceedings of the 9th Annual Meeting of the Society for Biological Therapy, Oct 26-20, 1994
(44) Fishbein GE, McClay E, Berd D, et al: A postsurgical adjuvant trial of CEA immunization in patients with Dukes C and D colorectal cancer: a pilot study. Vaccine Res 1:123-128, 1992
(45) Rosenberg SA, Yannelli JR, Yang JC, et al: Treatment of patients with metastatic melanoma with autologous tumor-infiltrating lymphocytes and interleukin 2. J Natl Cancer Inst 86:1159-1166, 1994
(46) Gavioli R, Kurilla MG, DeCampos-Lima PO, et al: Multiple HLA-A11-restricted cytotoxic T-lymphocyte epitopes of different imunogenicities in the Epstein-Barr virus-encoded nuclear antigen 4. J Virol 67:1572-1578, 1993
(47) Oldstone MB, Tishon A, Geckeler R, et al: A common antiviral cytotoxic T-lymphocyte epitope for diverse major histocompatibility complex haplotypes: implications for vaccination. Proc Natl Acad Sci U S A 89:2752-2755, 1992
(48) Hodge JW, Abrams S, Schlom J, et al: Induction of antitumor immunity by recombinant vaccinia viruses expressing B7-1 or B7-2 costimulatory molecules. Cancer Res 54:5552-5555, 1994

Notes

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