Strategies for cancer therapy using carcinoembryonic antigen vaccines

Heidi Hörig, Freddy A. Medina, William A. Conkright and Howard L. Kaufman

Advances in molecular biology and immunology have renewed interest in the development of vaccines for the treatment or prevention of cancer. Research over the past 10 years has focused on the identification of suitable tumour antigens to use as targets for a variety of vaccine strategies. Carcinoembryonic antigen (CEA) was one of the first tumour antigens described, and is commonly expressed by a wide range of adenocarcinomas. Recent studies have identified several human-leukocyte-antigen-restricted epitopes (short peptides) within the CEA protein that can be recognised by human T lymphocytes (T cells). Although CEA-expressing tumour cells are generally weakly recognised by the immune system, several new strategies have been used to enhance immune responses against CEA. This includes using antibodies directed against CEA; inserting the CEA gene into recombinant viruses and bacteria as viral and bacterial vaccines; pulsing the CEA protein, peptides, DNA or RNA onto dendritic cells (specialised antigen-presenting cells); and combining CEA vaccines with cytokines or co-stimulatory molecules to increase vaccine effectiveness. Other factors that might be important in establishing systemic immunity against CEA are the dose, route, timing, and choice of vector and adjuvants for vaccine administration. Further research in understanding the fundamental processes involved in tumour-cell recognition by the immune system, better animal models, and improved clinical trial designs will help to define the full potential of CEA as a target for cancer vaccine development.

Howard L. Kaufman (corresponding author), Assistant Professor, Departments of Surgery and Microbiology & Immunology, Albert Einstein College of Medicine, New York, NY, USA. Tel: +1 718 430 3517; Fax: +1 718 430 3099; E-mail: kaufman@aecom.yu.edu

Heidi Hörig, Instructor, Department of Microbiology & Immunology, Albert Einstein College of Medicine, New York, NY, USA. Tel: +1 718 430 3520; Fax: +1 718 430 3099; E-mail: horig@aecom.yu.edu

Freddy A. Medina, Sue Golding Graduate Student, Department of Microbiology & Immunology, Albert Einstein College of Medicine, New York, NY, USA. Tel: +1 718 430 3519; Fax: +1 718 430 3099; E-mail: fmedina@aecom.yu.edu

William A. Conkright, Research Associate, Department of Surgery, Albert Einstein College of Medicine, New York, NY, USA. Tel: +1 718 430 3678; Fax: +1 718 430 3099

Laboratory website: http://www.aecom.yu.edu/cancer/kaufman

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History of tumour vaccines

Vaccination has become standard procedure for the prevention of numerous infectious diseases. The application of vaccines to other diseases, such as cancer, is now possible owing to advances in molecular engineering and a better understanding of tumour immunology. The concept of vaccines for cancer treatment is not new and was suggested nearly 100 years ago, when William Coley reported that inoperable sarcomas underwent regression after patients developed erysipelas, a severe bacterial skin infection (Ref. 1). Over the years, many attempts have been made to generate effective cancer 'vaccines' from mixtures of tumour cells and infectious particles (so-called Coley's toxins) without much success. During this time, studies of transplantable tumours in animals established the feasibility of tumour rejection through immune-mediated mechanisms. These studies suggested that tumour cells expressed unique antigens (i.e. antigens that were not found on normal cells). These antigens, under appropriate conditions, could be recognised by components of the immune system. Further research identified many of the antigens that induced tumour rejection as normal self-proteins. There are many reasons why self-proteins might be recognised by the immune system, including the presence of mutations in the coding regions of the protein, unusually high levels of the protein, and abnormal patterns of glycosylation of the protein. The awareness that T lymphocytes (T cells) are significant mediators of tumour rejection has focused attention on the isolation of antigens that are specifically recognised by T cells. T cells recognise antigens as smaller fragments of proteins, so-called epitopes, only after their intracellular degradation and presentation on the cell surface, where they are bound to the major histocompatibility complex [MHC, or human leukocyte antigen (HLA) in humans]. The first T-cell-specific tumour antigen was derived from malignant melanoma cells; subsequently, many other tumour antigens in a variety of tumours have been found to possess T-cell-specific epitopes.

History of CEA

CEA was one of the first tumour-associated antigens to be identified and has been well characterised. CEA is an oncofetal glycoprotein, which is found at high levels in the fetal colon and at lower levels in the normal adult colonic epithelium. CEA occurs at abnormally high levels in several benign disorders and in some malignant tumours, including those of the stomach, small intestine, colon, rectum, pancreas, liver, breast, ovary, cervix and lung (Refs 2, 3). Recently, several T-cell epitopes within CEA that are recognised by human T cells have been described (Refs 4, 5, 6). Several different strategies are now using vaccination to target CEA, and clinical trials have started to yield interesting findings. In this review, we have discussed the rationale for using CEA as a target for vaccination, some of the various strategies for enhancing vaccination against CEA, and some of the problems that need to be solved before CEA vaccines can be considered standard therapy. The current status of clinical trials and new animal models have also been reviewed. Although more research is necessary, successful vaccination against CEA could affect many individuals who have cancer and even more individuals who are at risk of developing cancer. Thus far, CEA appears to be a promising antigen for vaccine therapy; however, further studies are required to define the best strategy for the clinical application of CEA-directed vaccines.

Biology of CEA

CEA is a 180-kD glycoprotein that occurs at high levels in colon epithelial cells during embryonic development. Levels of CEA are significantly lower in colon tissue of adults, but can become elevated when inflammation or tumours arise in any endodermal tissue, including in the gastrointestinal tract, respiratory tract, pancreas and breast. CEA was originally isolated from a colon carcinoma specimen in 1965 (Ref. 7). The construction of monoclonal antibodies against CEA allowed the detection of the overexpression of CEA protein in a variety of adenocarcinomas, including gastric, pancreatic, small intestine, colon, rectal, ovarian, breast, cervical and non-small-cell lung cancers (Refs 2, 3). Currently, ~500 000 individuals are diagnosed with CEA-producing tumours each year in the USA alone (Ref. 8). CEA is also expressed by epithelial cells in several non-malignant disorders, including diverticulitis, pancreatitis, inflammatory bowel disease, cirrhosis, hepatitis, bronchitis and renal failure and also in individuals who smoke (Ref. 9). This fact has made it difficult to use serum CEA determination as a sensitive method for cancer screening. However, serum CEA levels have been useful in monitoring individuals for the recurrence of cancer (Ref. 10).

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In 1986, the gene that encodes human CEA was localised to chromosome 19, and subsequently cloned (Ref. 11). In humans, the CEA gene encodes a messenger RNA (mRNA) that is 3100 base pairs long and translates to a protein that has a molecular weight of 70 kD. The additional weight of the protein is provided by an extensive pattern of carbohydrates that are added by glycosylation enzymes, leading to a final weight of 180 kD. The structure of CEA protein includes an N-terminal sequence followed by three disulphide-linked repeats of 178 amino acids, and a hydrophobic C-terminal domain (Fig. 1). This structure is similar to that of the immunoglobulins, and has established CEA as a member of the superfamily of immunoglobulin genes (Ref. 12). A unique feature of CEA is that it is linked via lipid into the membrane, through a glycosylphosphatidylinositol moiety, making it distinct from other members of the CEA family (Ref. 13). Several other antigens are closely related to CEA, including the non-specific cross-reacting antigen (NCA), biliary-specific glycoprotein (BGP), CEA gene family member CGM-6 and pregnancy-specific glycoproteins (Ref. 14). Some of these represent separate species, whereas others may be splice variants of CEA. Currently, 29 separate genes have been identified as coding a CEA-related gene product, and most of these genes are located on the long arm of chromosome 19 (Refs 15, 16, 17).
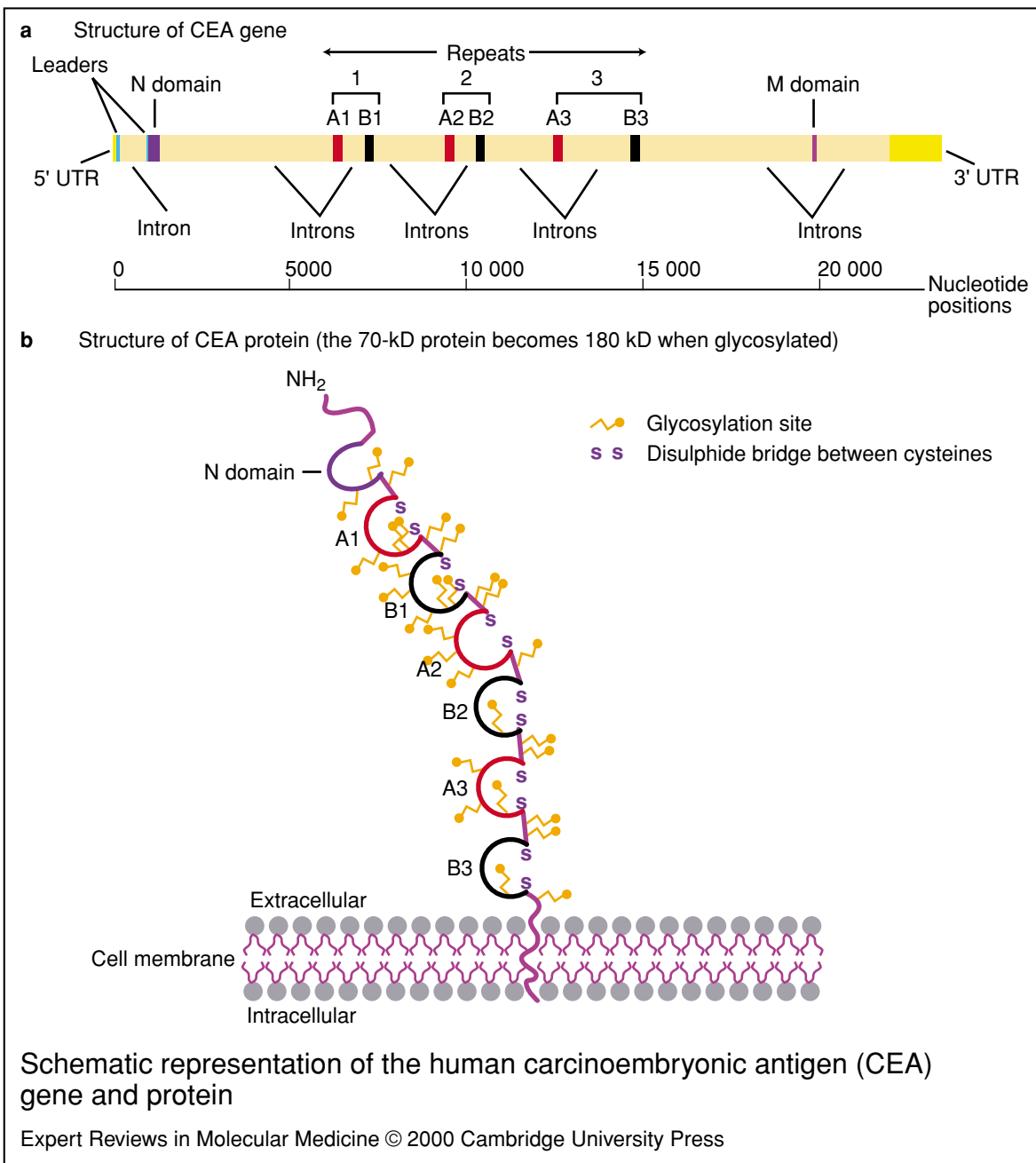
The function of CEA in normal colon epithelial cells and in tumour cells is not entirely clear. Many members of the immunoglobulin gene family serve as recognition markers, and this might be true for CEA as well. Studies have reported that CEA localised on the cell surface of colon tumours and other cells can act as a homotypic adhesion molecule, resulting in the aggregation of CEA-expressing cells (Ref. 18). Furthermore, although CEA is produced at low levels in normal colonic epithelial cells in adults, the pattern of localisation differs from that observed in most colon tumour cells or in the developing embryonic colon. In normal colonocytes, CEA is localised only at the luminal surface of the cells, whereas in tumour cells, it is found in a disordered pattern throughout the cell membrane (Ref. 18). Thus, current models suggest that CEA promotes the spatial orientation of colon epithelial cells to one another and to the surrounding matrix during embryonic development of the colon, and helps maintain the integrity of the luminal epithelium in the adult colon. The altered pattern of localisation in tumour cells may help to disrupt the intercellular adhesion of colonocytes, resulting in the disorganised growth and movement of malignant cells. CEA may also be involved in the enhancement of metastatic disease. Elevated levels of CEA in the serum have been shown to correlate with an increased incidence of liver metastases, and this may be due to adhesion between circulating CEA in the liver and CEA bound to metastatic tumour cells (Ref. 19). This may explain the high incidence of hepatic metastases in those patients who have primary tumours that express CEA.

Several different lines of investigation have suggested the possibility that CEA can serve as an antigenic target for eliciting anti-cancer immune responses. Adaptive immune responses to any antigen can be broadly characterised by the production of specific antibodies (i.e. humoral immunity) or the generation of antigen-specific T cells (i.e. cellular immunity). Shortly after the discovery of CEA protein, several groups sought to determine whether individuals who had colon cancer developed anti-CEA antibodies during the course of their disease (Refs 20, 21). Some groups did not find significantly elevated titres of such antibodies; however, others did find evidence that antibody responses to CEA occurred in some individuals. The reasons for this discrepancy might relate to the presence of circulating antigen–antibody complexes, making it difficult to detect the antibodies, especially using the technology that was available at the time (Ref. 22). The potential for CEA to elicit T-cell responses was first suggested by the observation that individuals who had colon cancer often exhibited a delayed-type hypersensitivity (DTH) response to purified CEA protein (Ref. 23). More recently, recombinant vaccinia viruses expressing CEA were administered to cancer patients, and CEA-specific T cells were subsequently cloned from these patients, demonstrating that T cells can recognise CEA (Ref. 4). Several independent groups have now reported the existence of multiple epitopes within CEA that are recognised by human T cells that bind to various HLA class I molecules (Refs 4, 5, 6).

Strategies for CEA cancer vaccine development

Two lines of evidence have supported the use of CEA as a target for vaccine development:

a Structure of CEA gene

b Structure of CEA protein (the 70-kD protein becomes 180 kD when glycosylated)

Schematic representation of the human carcinoembryonic antigen (CEA) gene and protein

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Figure 1. Schematic representation of the human carcinoembryonic antigen (CEA) gene and protein
(a) The CEA gene is encoded by a segment of DNA that is 3100 base pairs in length and is derived from eight exons (N domain, A1–A3, B1–B3 and M domain; Ref. 17). (b) The CEA protein product contains a leader sequence and three highly conserved repeat domains (1–3), each comprising 178 amino acids. Each of these three repeat domains can be further divided into two sub-domains (A and B), which share significant sequence homology. Each domain contains four cysteine residues at similar positions, which pair up to form A and B 'loops' stabilised by disulphide bridges between the cysteines. (a) The domains and sub-domains in the CEA gene correspond to the labelled domains of the mature protein shown in (b). The CEA protein consists of 668 amino acids, and has a configuration that is similar to that of other members of the immunoglobulin gene superfamily. The protein extends out from the cell membrane into the extracellular space, and is anchored through a hydrophobic C-terminal region (the M domain; Ref. 13). Most of the final molecular weight of CEA is provided by N-linked glycosylation, which occurs at the sites indicated in (b) **(fig001hka)**.

the high level of expression of the CEA gene in many different human tumours, and the emerging information about the molecular biology and immunology of CEA. The experimental generation of monoclonal antibodies against CEA paved the way for a variety of diagnostic and therapeutic approaches for cancer therapy based on the detection and targeting of CEA (see next section; Ref. 24). These have included the direct, in vivo use of anti-CEA monoclonal antibodies, either alone or coupled with radioisotopes or cellular toxins, and also the use of anti-idiotype antibodies. Strategies that target CEA-reactive T cells have also been proposed, including the use of specific HLA-restricted peptides derived from CEA, recombinant viruses and bacteria expressing CEA peptides or proteins, and the pulsing of CEA onto antigen-presenting cells (APCs). Dendritic cells are the most potent type of APC; thus, dendritic cells loaded with CEA peptides, DNA or RNA have been used to stimulate T cells (Ref. 25). Although all of these represent logical approaches for the treatment of CEA-expressing tumours, the optimal therapeutic vector(s), dose, routes of delivery and schedule remain to be defined. However, clinical trials of such vaccines have begun, and will likely provide important insights that will help to resolve these issues.
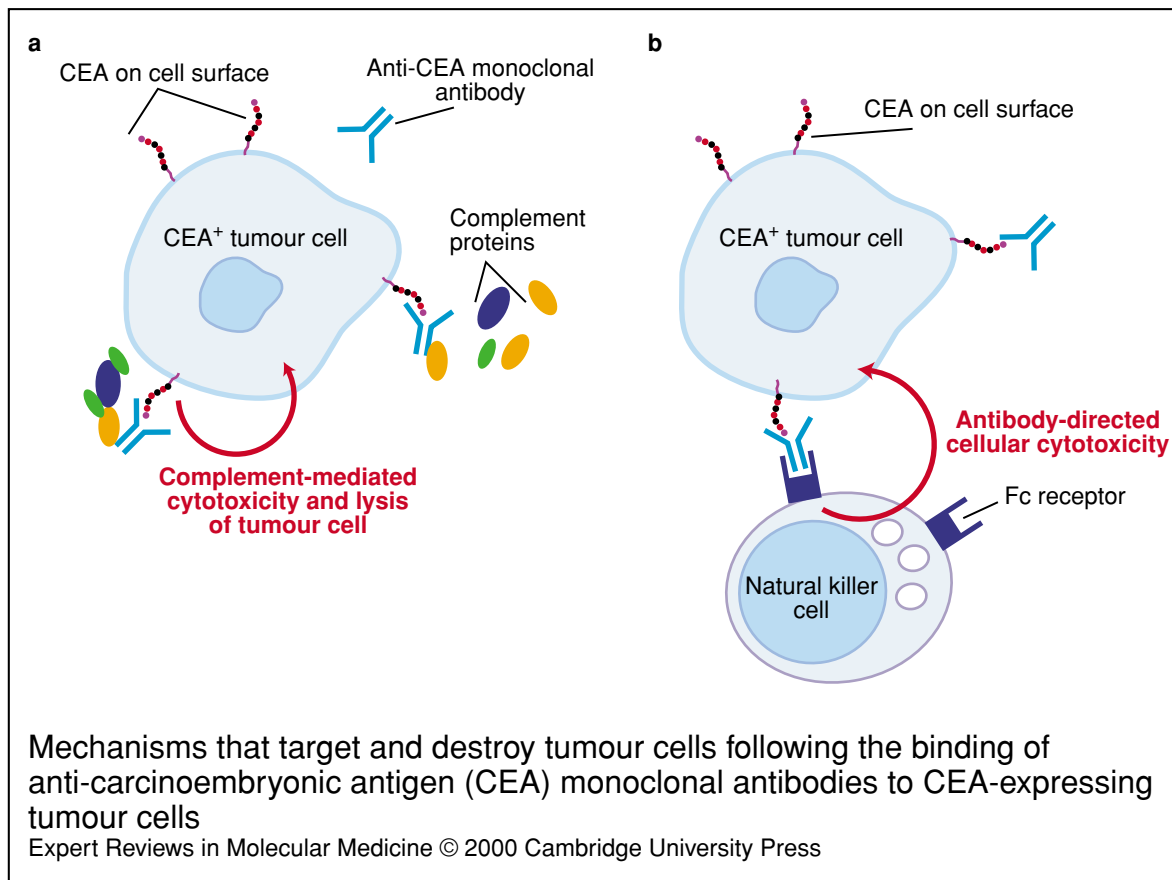
Monoclonal-antibody therapy
Monoclonal antibodies directed against CEA were initially used for diagnostic purposes, including the immunohistochemical staining of tissue specimens and the localisation of disease in vivo. The coupling of a radioactive isotope to the antibody greatly enhanced the effectiveness of using monoclonal antibodies for detecting potential sites of disease (Ref. 26). The use of antibody-targeted therapeutics for cancer treatment has shown that tumour-cell lysis can be initiated by immune-mediated mechanisms. Antibodies can also be used for the direct delivery of cytotoxic molecules such as radionuclides, toxins or chemotherapy agents to the site of an established tumour.

Unconjugated antibodies
The discovery that anti-CEA antibodies can be used to detect CEA-expressing tumour cells hinted that they could also be used to mediate the rejection of tumour cells through immune mechanisms. When anti-CEA antibodies bind to

the surface of a tumour cell, several pathways are activated, which can result in the destruction of an antibody-marked cell (Fig. 2). The presence of bound antibody can activate the complement cascade, leading to cell lysis (complement-mediated cytotoxicity). Another pathway that might be more relevant to tumour cells is the initiation of antibody-directed cellular cytotoxicity (ADCC). This cytotoxic reaction occurs when the Fc portion of an antibody binds to and triggers Fc-receptor-bearing natural killer cells to release cytotoxic granules that lyse cells that are coated with the antibody (Ref. 27). However, these anti-tumour effects depend on the presence of CEA on the surface of targeted tumour cells, and because CEA is often found in a heterogeneous pattern, it is difficult to eradicate all of the cells within a tumour mass. Furthermore, the anti-CEA antibody must be able to circulate throughout the body and penetrate solid tumours. This often cannot occur owing to an inadequate blood supply to the tumour. Because many of the monoclonal antibodies that are developed for in vivo clinical use are derived from mice, strong human anti-mouse antibodies (HAMA) can be induced in the patient upon repeated use of the monoclonal antibody; thus, the mouse monoclonal antibody is eliminated before it reaches the tumour.

Using another approach, anti-idiotype antibodies can be used to either elicit or amplify an antigen-specific immune response. For example, immunisation with CEA protein induces the production of Ab1 antibodies. The antigen-binding site of an Ab1 antibody contains a hypervariable complementarity-determining region, which is complementary to the epitope on the antigen that is bound by the antibody. This region is also known as the idiotype, and can induce the production of host antibodies. Immunisation with these idiotypes generates a series of anti-idiotype antibodies, known as Ab2 antibodies, which can resemble some of the epitopes of the original antigen. Thus, Ab2 antibodies then induce the production of anti-anti-idiotype antibodies (Ab3 antibodies), which can specifically bind to the original antigen (Ref. 28). The experimental in vivo use of an Ab2 antibody generated against CEA protein in mice has been described; Ab2-immunised mice were protected against challenge with lethal doses of CEA-expressing tumours (Ref. 29). Clinical trials of this antibody have been conducted, and most patients

Mechanisms that target and destroy tumour cells following the binding of anti-carcinoembryonic antigen (CEA) monoclonal antibodies to CEA-expressing tumour cells
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Figure 2. Mechanisms that target and destroy tumour cells following the binding of anti-carcinoembryonic antigen (CEA) monoclonal antibodies to CEA-expressing tumour cells. (a) Anti-CEA monoclonal antibody binds to CEA expressed on the surface of the tumour cell, leading to the accumulation of complement proteins, which are circulating in the intercellular space. The complement system consists of proteolytic enzymes, regulatory and inflammatory proteins and peptides, cell-surface receptors, and other proteins, all of which interact in a cascade of events that results in the lysis of tumour cells. (b) Binding of natural killer cells via their Fc receptor to the Fc portion of bound anti-CEA antibodies can also activate natural killer cells to lyse tumour cells through a process called antibody-directed cellular cytotoxicity (ADCC) **(fig002hka)**.

did develop Ab3 responses that were specific for CEA (Ref. 30). Furthermore, one of four patients tested also developed T-cell responses to CEA, although no objective clinical responses were observed (Ref. 31).

Antibody conjugates
Another method of using monoclonal antibodies for cancer therapy is to conjugate them to a radionuclide, which can deliver damaging radiation to the vicinity of the tumour (Ref. 32). The advantage of this approach is that targeting a single cell expressing CEA can also lead to the death of nearby tumour cells that are not expressing CEA, owing to a by-stander effect. A similar approach can be used to deliver

chemotherapeutic drugs that are known to be toxic to the tumour cells. This is accomplished by conjugating the chemotherapeutic drug to the anti-CEA antibody. The administration of the conjugated antibody results in the accumulation of toxic drug at the site of the tumour rather than in normal tissues (Ref. 33). Yet another approach is the construction of genetically modified monoclonal antibodies that are fused with cellular toxins, such as ricin (Ref. 34). All of these specialised antibodies can have a by-stander effect, avoiding the problem of heterogeneous CEA expression, but because of the size of the antibody conjugates, delivery and HAMA responses are still problematic. The use of humanised monoclonal antibodies, or chimaeric

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antibodies that contain only the murine variable regions that interact with antigen combined with human Fc portions, seems to avoid or reduce the HAMA response. However, the delivery of such antibodies to the tumour site still remains a problem (Ref. 35). The variable region of the antibody is used to target the cells, and is contained within the Fab portion of the antibody molecule. Because only the Fab fragment is necessary for antigen recognition, smaller antibody fragments containing the Fab protein can be used for targeting tumour cells and enhancing delivery to sites of tumour growth.

CEA-derived peptides

T cells appear to play a major role in tumour rejection after vaccination. Antibodies recognise their antigens by the three-dimensional structure of a single antigenic determinant, the so-called epitope. However, T cells recognise antigen only after the antigen has been processed into smaller linear peptide fragments, which are also known as epitopes. These epitopes are loaded onto specific molecules called major histocompatibility complex (MHC) proteins, so called because they are known to mediate transplantation rejection. MHC class I molecules are found on all nucleated cells, and are recognised by the T-cell receptors (TCRs) of CD8^{+} T cells. MHC class II molecules are mainly expressed by APCs, and are recognised by CD4^{+} T cells. Figure 3 shows our current understanding of how tumour antigens, such as CEA, can elicit an immune response: CEA protein, or peptides, which may have been derived from secreted CEA protein, or as a result of cellular necrosis or apoptosis of tumour cells, are engulfed by an APC. The CEA protein or peptides are processed and small peptide fragments of 12–16 amino acids in length are presented through the MHC class II pathway to helper CD4^{+} T cells. These cells stimulate an immune response by releasing local cytokines [e.g. interleukin 2 (IL-2), interleukin 12 (IL-12) and interferon γ (IFN-γ)], and can further prepare the APC for the activation of CD8^{+} T cells. Activated CD8^{+} T cells include cytotoxic T lymphocytes (CTLs) that can recognise CEA peptides of 8–11 amino acids in length that are displayed by the MHC class I molecules on the surface of APCs and, subsequently, on tumour cells. Once the CTLs are activated, the local release of perforins and granzymes destroys the CEA-expressing tumour cells.

Identification of CEA epitopes

Several experimental approaches have been used to identify CEA epitopes that are presented by MHC class I molecules to CD8^{+} T cells. Thus far, the most commonly used approach has been the identification of a putative peptide sequence by using a computer to predict binding affinity to specific MHC class I molecules. This is now easy because the amino acid sequence of the CEA protein has been determined and all nine potential amino acid sequences can be quickly modelled. The peptide groove of an MHC class I molecule normally binds short peptide fragments that comprise 8–10 amino acids, and because the three-dimensional structure of several MHC molecules is known, the computer models can predict the potential peptides that would be expected to bind with high affinity (Ref. 36). These peptides can be synthesised and tested in vitro for their actual binding affinity for the MHC molecule and for recognition by specific CTLs (Refs 37, 38, 39).

The above-described method was used to isolate the first HLA-restricted CEA peptide, namely carcinoembryonic-antigen-associated peptide 1 (CAP-1). CAP-1 peptide binds to the HLA-A2 complex, and has been used to generate T-cell lines (i.e. a mixed T-cell population that responds to CEA) from cancer patients who have been vaccinated with recombinant vaccinia virus expressing CEA (Ref. 4). A T-cell clone (i.e. a single, genetically identical T-cell population that recognises CEA) derived from one of these patients has been shown to lyse target cells that contain CAP-1 and the HLA-A2 complex. To date, several CEA peptides that specifically bind to known HLA molecules have been identified and have elicited T-cell responses (Table 1). In theory, these peptides can be used to immunise individuals who express the same HLA molecule.

Until recently, CD4^{+} T cells have received far less attention in tumour immunology. This is due, in part, to less being known about MHC class II structures, and to the fact that most tumour cells do not express MHC class II molecules. However, increasing knowledge about the role of CD4^{+} T cells in orchestrating antigen-specific immune responses and the identification of an increasing number of MHC-class-II-restricted tumour antigens emphasises the importance of these epitopes (Refs 40, 41, 42, 43, 44). Although several MHC class II epitopes have been described for melanoma antigens, there have not been reports of MHC class II CEA peptides yet. The peptides
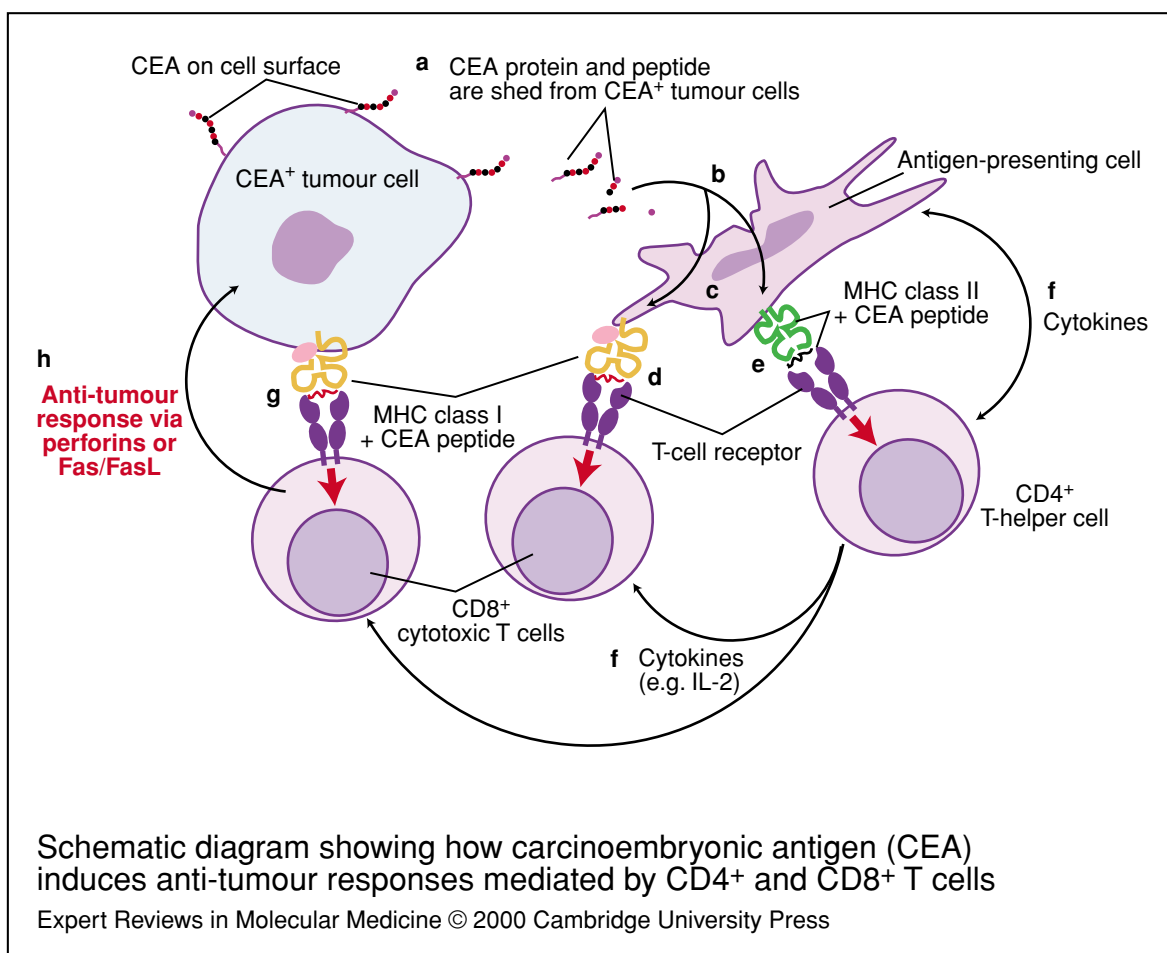
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Schematic diagram showing how carcinoembryonic antigen (CEA)
induces anti-tumour responses mediated by CD4$^+$ and CD8$^+$ T cells

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Figure 3. Schematic diagram showing how carcinoembryonic antigen (CEA) induces anti-tumour responses mediated by CD4$^+$ and CD8$^+$ T cells. (a) CEA proteins derived from tumour cells are shed and (b) taken up by antigen-presenting cells (APCs) including dendritic cells. (c) The APC processes the antigen into smaller peptide fragments and (d) presents these via major histocompatibility complex (MHC) class I molecules to CD8$^+$ T cells and (e) via MHC class II molecules to CD4$^+$ T cells. CEA is recognised in the form of processed peptides by T-cell receptors (TCRs). (f) The recognition of CEA by CD4$^+$ T cells leads to the release of cytokines [e.g. interleukin 2 (IL-2)] and helps to further activate CD8$^+$ T cells. (g) CD8$^+$ T cells activated specifically against CEA peptides can then directly target tumour cells that are expressing CEA peptides on MHC class I molecules. (h) The cytotoxic T-cell response is mediated either by perforins (leading to the lysis of tumour cells) or by receptors [e.g. Fas or Fas ligand (FasL)] that are involved in programmed cell death (apoptosis) **(fig003hka)**.

that bind to MHC class II molecules are also longer than the short MHC class I peptides, and often overhang the binding site of MHC class II molecules, making it more difficult to predict binding affinity accurately.

Modified CEA peptides
CEA is a self-antigen and is generally considered to be weakly immunogenic. One reason for this might be that CEA peptides bind to MHC molecules or TCRs with low affinity, thus decreasing peptide recognition by T cells. One

method for enhancing recognition is to alter the affinity of the peptide for MHC molecules or TCRs by amino acid substitutions of peptide anchor residues or non-anchor residues, respectively (Fig. 4). This strategy can be applied to any known peptide epitope and may be particularly helpful for increasing the immunogenicity of self-antigens. Modifications in the anchor binding residues have resulted in higher affinity binding and better T-cell responses for several melanoma antigens (Ref. 45). The CEA peptide CAP-1 was modified by replacing an asparagine residue (N)

**Table 1. Carcinoembryonic antigen (CEA) peptides that are recognised
by human CD8$^+$ T cells (tab001hka)**

Peptide (CEA amino acid residues)	Sequence	HLA restriction	Ref.
CAP-1$_{571–579}$	YLSGANLNL	A2	4
CAP-1-6D[a]	YLSGADLNL	A2	46
CEA$_{61–69}$	HLFGYSWYK	A3	5
CAP$_{570–579}$	SYLSGANLNL	A24	[b]
CAP$_{268–278}$	QYSWFVNGTF	A24	6
CAP$_{318–327}$	TYACFVSNL	A24	6

[a] See section entitled 'Modified CEA peptides'
[b] Jeffrey Schlom (National Cancer Institute, Bethesda, USA, pers comm.)
Abbreviations used: CAP = CEA-associated peptide; HLA = human leukocyte antigen

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with an aspartic acid residue (D) at position 6 (Ref. 46). The resulting 'agonist' peptide, designated CAP-1-6D, was recognised by T cells more efficiently than the native CAP-1. Although modified peptides can be used as therapeutic vaccines, the CAP-1-6D peptide has yet to be tested in clinical trials.
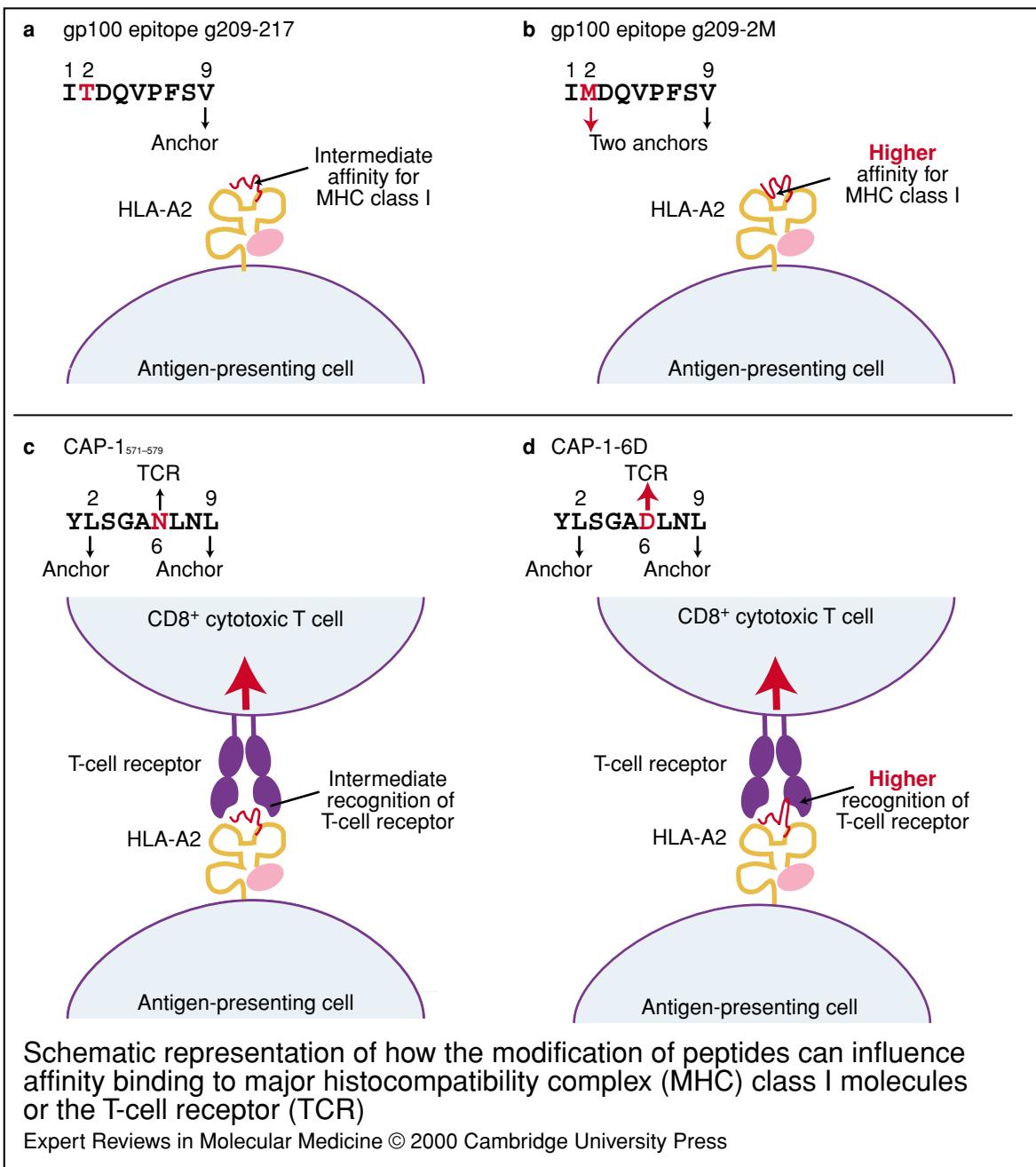
Recombinant CEA protein
MHC-class-I-restricted CEA peptides have been identified and have been shown to generate CEA-specific T-cell responses; however, such peptides can be used in a clinical setting to treat only those patients whose MHC type is analogous to that of the peptide. Additionally, effective anti-tumour immune responses might depend on the presentation of multiple CEA epitopes through all available MHC molecules expressed in each individual. This increase in peptide diversity can be accomplished by delivering the full-length protein to APCs. Sources of CEA protein include preparations from either tumour biopsy specimens and/or supernatants derived from tumour-cell lines, both of which can contain contaminants. Recombinant baculoviruses (insect viruses) expressing the full-length human CEA gene can also be used (Ref. 47). Studies have shown that better humoral and cellular immune responses were elicited in mice by priming (i.e. administering a first vaccination) with recombinant vaccinia virus containing CEA followed by boosting (i.e. administering a second vaccination) with recombinant CEA protein

than by vaccination with either virus or protein alone (Ref. 47).

Intramuscular injection of recombinant baculovirus containing human CEA protein has been evaluated in a clinical trial involving five individuals who had metastatic breast cancer that responded to hormonal therapy. Two of the patients produced both lymphoproliferative responses (i.e. T-cell stimulation and growth) to recombinant CEA protein and strong DTH responses (as revealed by a skin test) after immunisation (Ref. 48). In another clinical trial, patients who had colorectal carcinoma were immunised using either recombinant baculovirus containing human CEA alone or in combination with the cytokine granulocyte-macrophage colony-stimulating factor (GM-CSF; see later section entitled 'Cytokines'). All six of the patients who received the combination treatment showed early CEA-specific T-cell proliferation after immunisation, whereas only two of the six patients who were immunised with recombinant baculovirus containing CEA without GM-CSF developed an anti-CEA T-cell response after multiple vaccinations (Ref. 49). These studies provide good evidence for the use of recombinant CEA protein as a boost following primary viral immunisation, or for its use in combination with immune-stimulatory cytokines.

DNA vaccines
DNA vaccines consist of a bacterial plasmid that contains genes (e.g. pathogens, allergens or

a gp100 epitope g209-217

1 2   9
I**T**DQVPFSV
↓
Anchor

HLA-A2

Intermediate affinity for MHC class I

Antigen-presenting cell

b gp100 epitope g209-2M

1 2   9
I**M**DQVPFSV
↓
Two anchors

Higher affinity for MHC class I

HLA-A2

Antigen-presenting cell

c CAP-1$_{571-579}$

TCR
2   ↑   9
YLSGA**N**LNL
↓   6   ↓
Anchor   Anchor

CD8$^+$ cytotoxic T cell

T-cell receptor

HLA-A2

Intermediate recognition of T-cell receptor

Antigen-presenting cell

d CAP-1-6D

TCR
2   ↑   9
YLSGA**D**LNL
↓   6   ↓
Anchor   Anchor

CD8$^+$ cytotoxic T cell

T-cell receptor

HLA-A2

Higher recognition of T-cell receptor

Antigen-presenting cell

Schematic representation of how the modification of peptides can influence affinity binding to major histocompatibility complex (MHC) class I molecules or the T-cell receptor (TCR)

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Figure 4. Schematic representation of how the modification of peptides can influence affinity binding to major histocompatibility complex (MHC) class I molecules or the T-cell receptor (TCR). (a) Substitution of a (potential anchor) residue (the second amino acid in this case) in the gp100 epitope g209-217 (ITDQVPFSV). Replacing a threonine residue (T) with a methionine residue (M) results in the modified g209-2M peptide (IMDQVPFSV). (b) This change alters the binding affinity of the peptide to the HLA-A2 molecule, increasing the affinity of the peptide for the MHC (because of its second anchor position for the MHC), and stabilising the complex (Refs 45, 90). This leads to an increased recognition of the MHC–peptide complex by the TCR. (c) Substitution of an amino acid residue that is not an anchor for MHC can, instead, alter the recognition of MHC-bound peptide for the TCR. For example, replacing an asparagine residue (N) at position 6 of the CAP-1$_{571-579}$ peptide (YLSGANLNL) with an aspartic acid residue (D) results in the modified CAP-1-6D peptide (YLSGADLNL). (d) This modification does not alter the binding affinity of the peptide to the HLA-A2 molecule, but rather increases the recognition of the MHC–peptide complex by the TCR (Ref. 46) **(fig004hka)**.

tumour antigens) that are under the control of a strong eukaryotic promoter. The DNA is usually taken up into host cells, where the encoded antigen is produced and processed via both MHC class I and II pathways, inducing CD8^{+} and CD4^{+} T-cell responses. In contrast to viral vaccines, DNA vaccines are relatively simple to produce; moreover, they do not inhibit the immunological responses (e.g. downregulate the MHC class I pathway) that are often associated with viral infections. Naked DNA (i.e. plasmid DNA in saline) has been used for vaccination; this resulted in stable expression after intramuscular injection and the induction of both cellular and humoral (antibody) immune responses (Refs 50, 51). The immune mechanisms involved are only partly understood. It has been suggested that nucleic acid might be taken up selectively by macrophages and/or APCs in the muscle. These activated macrophages then migrate to draining lymph nodes, where they stimulate naive T cells (Ref. 52).

A plasmid that encoded the full-length human CEA has been tested by injecting it intramuscularly into mice; both CEA-specific humoral and cell-mediated immune responses were induced. This DNA vaccine also protected mice from a challenge with CEA-expressing colon tumours (Ref. 53). The application of DNA vaccines in humans raises several concerns; one of these concerns is the potential for such vaccines to induce anti-DNA antibodies, as observed in patients who have systemic lupus erythematosus (SLE). However, animal studies have shown that vaccination with purified DNA does not induce anti-DNA antibodies (Ref. 54). Clinical trials using CEA-encoded DNA vaccines are currently in progress and await further evaluation.

Dendritic cells
Dendritic cells are the most potent APCs and present antigen via the MHC class I and MHC class II pathways. The use of dendritic cells that have been pulsed (i.e. exposed for a short time to high concentrations) with specific antigens has been proposed as a means of generating more-effective antigen-specific T-cell responses (Ref. 55). The identification of several dendritic-cell growth factors, such as GM-CSF and interleukin 4 (IL-4), has permitted their in vitro expansion and activation (Refs 56, 57, 58). Populations of dendritic cells from individual patients (autologous dendritic cells) can be generated using isolated monocytes from peripheral blood; these cells can be expanded ex vivo, pulsed with an antigen and then re-administered to the same patient as a dendritic-cell vaccine. Various strategies for pulsing dendritic cells have been proposed, including the use of MHC-restricted peptides, DNA, RNA, recombinant viruses and tumour-cell lysates. In a Phase I study, patients who had advanced malignancies expressing CEA were vaccinated with dendritic cells that had been pulsed with the CEA peptide CAP-1. A minor clinical response was observed for one of the patients in the study, and disease progression was stabilised in another (i.e. there was no tumour growth following vaccination). No treatment-related toxicities were observed, demonstrating the feasibility and safety of this treatment method (Ref. 59). Dendritic cells that had been pulsed with a cocktail of melanoma peptides or a tumour lysate were used to treat patients who had advanced melanoma by injecting the cells into or near lymph nodes. Five patients out of 16 produced a clinical response to the vaccine, and two of the five responded completely (Ref. 60).

Another interesting approach used CEA-specific mRNA and total RNA derived from CEA-expressing tumour cells. The advantage of using CEA-specific mRNA is that RNA encodes multiple CEA epitopes for various HLA types; thus, patients can be immunised without the need for prior identification of their HLA type or the use of HLA-specific CEA epitope(s). Moreover, RNA can be extracted from very small amounts of tumour tissue and encodes the individual array of tumour antigens for that tumour. Studies that utilised autologous dendritic cells that had been pulsed with either CEA peptides or CEA RNA to stimulate isolated T cells from carcinoma patients and healthy donors showed that a CEA-specific CTL response could be elicited in vitro (Refs 25, 61, 62). Another method of generating immunogenic vaccines is to fuse whole tumour cells directly to dendritic cells, using an electrofusion technique. In a pilot study involving patients who had renal cell carcinoma, the administration of a fusion vaccine composed of autologous renal cell carcinoma cells fused to allogeneic dendritic cells produced a significant clinical response in seven of the 17 treated patients, four of which showed complete responses (Ref. 63).

Strategies for cancer therapy using carcinoembryonic antigen vaccines

Bacterial vaccines

The delivery of DNA that encodes tumour antigens to APCs can also be accomplished using live attenuated bacteria. The advantages of using bacteria as expression vectors for foreign antigens include improved antigen presentation, because some bacteria are engulfed (taken up) by phagosomes, resulting in the presentation of inserted antigens by both MHC class I and class II pathways. Bacteria also provide the requisite transcriptional and translational machinery for the expression of foreign genes. This may not be true for some viruses that depend on host-cell transcription factors [e.g. retroviruses (lentivirus)] or translation factors [e.g. poxviruses (vaccinia virus)]. In bacteria, post-translational glycosylation of encoded proteins might be problematic. However, as most vaccines aim to elicit a T-cell response that is dependent on the MHC class I or class II pathway, the presentation of peptides should not be restricted. Another advantage of bacterial vectors is that they are sensitive to antibiotics and can be more easily controlled after administration to patients. Animal studies have shown the therapeutic effectiveness of using recombinant bacterial vaccines for the treatment of model tumours; both CD4+- and CD8+-antigen-specific T-cell responses were generated (Ref. 64). Bacteria that are amenable to the expression of tumour antigens include *Bacillus Calmette-Guerin* (BCG), *Salmonella typhimurium* and *Listeria monocytogenes*. The BCG vaccine has been successfully used to prevent tuberculosis around the world. Vaccination can be given after birth and results in few severe complications, even in individuals who are infected with human immunodeficiency virus type 1 (HIV-1; Refs 65, 66). BCG possesses strong immune adjuvant activity, and has been used extensively in the treatment of superficial bladder cancers (Refs 67, 68). Several clinical trials utilising admixtures of BCG with autologous tumour cells or peptides have been completed but produced limited clinical responses (Refs 69, 70). Much interest has been created in using BCG as a vector for the expression of CEA because of its favourable immunological properties and the fact that it has already been used in a variety of clinical settings (Ref. 71). *Listeria* recombinants that encoded model antigens (e.g. β-galactosidase and influenza nucleoprotein) have been shown to be effective in tumour models in mice, eliciting antigen-specific T-cell immunity (Refs 72, 73). A recombinant *Salmonella* vaccine that encoded *Listeria* antigens was orally administered to mice and generated a humoral and cellular immune response (Ref. 74). Bacterial recombinants that express human tumour antigens have not yet been tested in clinical trials. Although promising, further research is needed to better characterise the effects of bacterial vaccines as agents for cancer therapy.

Recombinant viruses

Perhaps the best-studied vaccine development method involves the use of recombinant viruses. The most characterised viral system is that of the poxviruses, particularly vaccinia virus. Recombinant vaccinia viruses can accept a large insert of foreign DNA, replicate accurately, are easily engineered, allow post-translational modification of foreign proteins (e.g. glycosylation), stimulate strong immune responses, and have been extensively used in the human population to prevent smallpox (Refs 75, 76). The methods for constructing recombinant vaccinia viruses have been well described, and several different tumour antigens have now been placed into these viruses, including CEA (Refs 77, 78).

In a colon carcinoma model in mice, vaccinia virus expressing CEA was found to be effective in treating established CEA-expressing tumours, and was associated with the development of anti-CEA antibody titres and T-cell responses (Ref. 79). Interestingly, the vaccine was most effective in preventing the growth of CEA-bearing tumours in pre-immunised animals. The same vaccine has also been tested for safety and immunogenicity in a non-human primate model; toxicity was found to be minimal and the monkeys produced CEA-specific T-cell responses after vaccination (Ref. 80).

Several clinical trials using recombinant vaccinia vaccine containing the CEA gene to treat patients who had advanced CEA-expressing tumours have provided evidence that vaccination was safe even when high titres of virus were given; CEA-specific T-cell growth and cytotoxicity were also induced (Refs 4, 81). These early clinical trials were designed to determine the safety of vaccination and not to detect clinical responses. All of the patients who were treated had advanced metastatic tumours; furthermore, they had all been vaccinated with smallpox (vaccinia). This would be expected to reduce the chances of an

anti-tumour response, owing to pre-existing anti-vaccinia immunity (i.e. memory T cells and B cells) preventing adequate boosting with repeated doses of recombinant vaccinia virus (Ref. 82).

To circumvent the neutralising antibody responses induced by vaccinia virus vaccination, attention has now focused on the use of attenuated vaccinia viruses and non-replicating poxviruses, such as the avipoxviruses. The attenuated vaccinia strains, such as NYVAC and modified vaccinia virus Ankara (MVA), contain multiple gene deletions, which prevent the virus from replicating in mammalian cells (Ref. 83). Although avipoxviruses, including fowlpox and canarypox (ALVAC) virus, are pathogenic in birds, they are also unable to replicate in mammalian cells. However, they are able to elicit strong T-cell immune responses in both rodent models and humans (Ref. 84). These T-cell responses have not been accompanied by the induction of strong neutralising antibodies and have allowed repeated immunisations. An ALVAC virus expressing CEA has shown therapeutic effectiveness in a CEA tumour model in mice, and has been tested in human clinical trials (Refs 85, 86). In a Phase I trial of ALVAC virus expressing CEA, seven of nine patients who had advanced carcinoma showed an increased CEA-specific T-cell response after vaccination without any significant side-effects; however, objective anti-tumour responses were not seen (Ref. 86).

The safety of viral vaccines and the ability to generate CEA-specific T-cell responses has led to several novel approaches for improving the clinical effectiveness of the vaccines. This includes the addition of adjuvants, such as cytokines and co-stimulatory molecules (see later sections entitled 'Cytokines' and 'Co-stimulation of tumour-antigen-specific T cells'), to the treatment regimen, and combining different viruses in a 'prime and boost' strategy.

Strategies for enhancing CEA cancer vaccines

Several approaches for vaccine design have been presented; however, the results from clinical trials have thus far been disappointing. One reason might be the use of vaccines in patients who have advanced disease, because they are less likely to elicit a measurable and protective immune response. Although such individuals may be able to respond to common antigens (e.g.

influenza or tetanus), their response may be locally immunosuppressed at the tumour site. Thus, patients who have advanced cancers may be less likely to respond to vaccination against a tumour-associated antigen. Several strategies could be employed to improve the ability of CEA vaccines to induce immune responses, as outlined below.

Cytokines
Cytokines are a large family of pleiotropic (i.e. they can act on many different cell types) immune regulatory proteins that are broadly involved in cell growth and differentiation. The release of cytokines by activated T lymphocytes can regulate the type and extent of an immune response that occurs after vaccination; thus, cytokines have been extensively studied for their ability to help induce anti-tumour immunity. IL-2 was the first cytokine to be shown to induce tumour regression in an animal model (Ref. 87). IL-2 has been tested against a variety of human cancers, and has been shown to have therapeutic potential when administered intravenously as a single agent for metastatic melanoma and renal cell carcinoma. Complete responses have been observed in 7–10% of patients who had either metastatic melanoma or renal cell carcinoma, and an additional 8–10% of the patients exhibited an objective partial response (Ref. 88). This response rate is modest, but the responses are often quite durable, making IL-2 the treatment of choice for many such patients.

The mechanism of tumour rejection associated with IL-2 in patients is still controversial, but probably depends on the expansion of tumour-specific T cells. Because vaccines can induce T-cell responses, it seems logical that IL-2 could be used to amplify the initial response, improving the therapeutic effects of cancer vaccines. This has been confirmed experimentally in a mouse model, whereby IL-2 significantly augmented the anti-tumour responses of a vaccinia virus expressing CEA (Ref. 89). A clinical response rate of 42% has been demonstrated in patients who had metastatic melanoma following treatment with a modified melanoma peptide in incomplete Freund's adjuvant and systemic IL-2. These patients experienced an objective cancer regression of their metastases in the brain, lung, liver, lymph nodes and skin (Ref. 90). Future studies, using a combination of systemic IL-2 with various forms of CEA vaccines, are necessary to see if similar

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improved clinical responses can be documented for patients who have CEA-expressing tumours.

Recombinant viral vaccines that encode both tumour antigen and cytokine genes have been constructed. These have been designed to induce the local release of cytokine at the site of T-cell activation, and should limit the systemic toxicity usually induced by the intravenous administration of high doses of IL-2. As a preliminary test of this system, the *LacZ* gene, which encodes β-galactosidase (an enzyme), was used as model tumour antigen. In a mouse model, the co-expression of *LacZ* and IL-2 in vaccinia virus enhanced the treatment of β-galactosidase-expressing pulmonary metastases and increased β-galactosidase-specific CTL responses (Ref. 91). The co-expression of human tumour antigens and IL-2 in viral vaccines may also be applicable for human studies in the near future.

The combination of numerous other cytokines with antigen-specific vaccines has improved the effects of tumour treatment methods. IL-12 is a cytokine that is involved in the stimulation of natural killer cells and the differentiation of naive T cells. Thus, IL-12 can be considered as an important mediator of the effector phase of cellular immunity. In mice, IL-12 significantly improved the treatment of a tumour, which had been transduced with the β-galactosidase model antigen, when combined with a recombinant vaccinia virus encoding β-galactosidase (Ref. 92). Other cytokines that have been evaluated for their ability to augment tumour vaccines include GM-CSF, IFN-γ, tumour necrosis factor α (TNF-α), interleukin 3 (IL-3), IL-4 and interleukin 10 (IL-10). GM-CSF has been shown to promote the growth and activation of dendritic cells, thus improving the antigen presentation 'arm' of the immune system. Irradiated tumour cells that were transduced ex vivo with the GM-CSF gene have been used as an autologous cellular vaccine and increased the anti-tumour T-cell response (Ref. 93). Early clinical trials are now testing viral CEA vaccines in combination with the local administration of GM-CSF (Ref. 94).
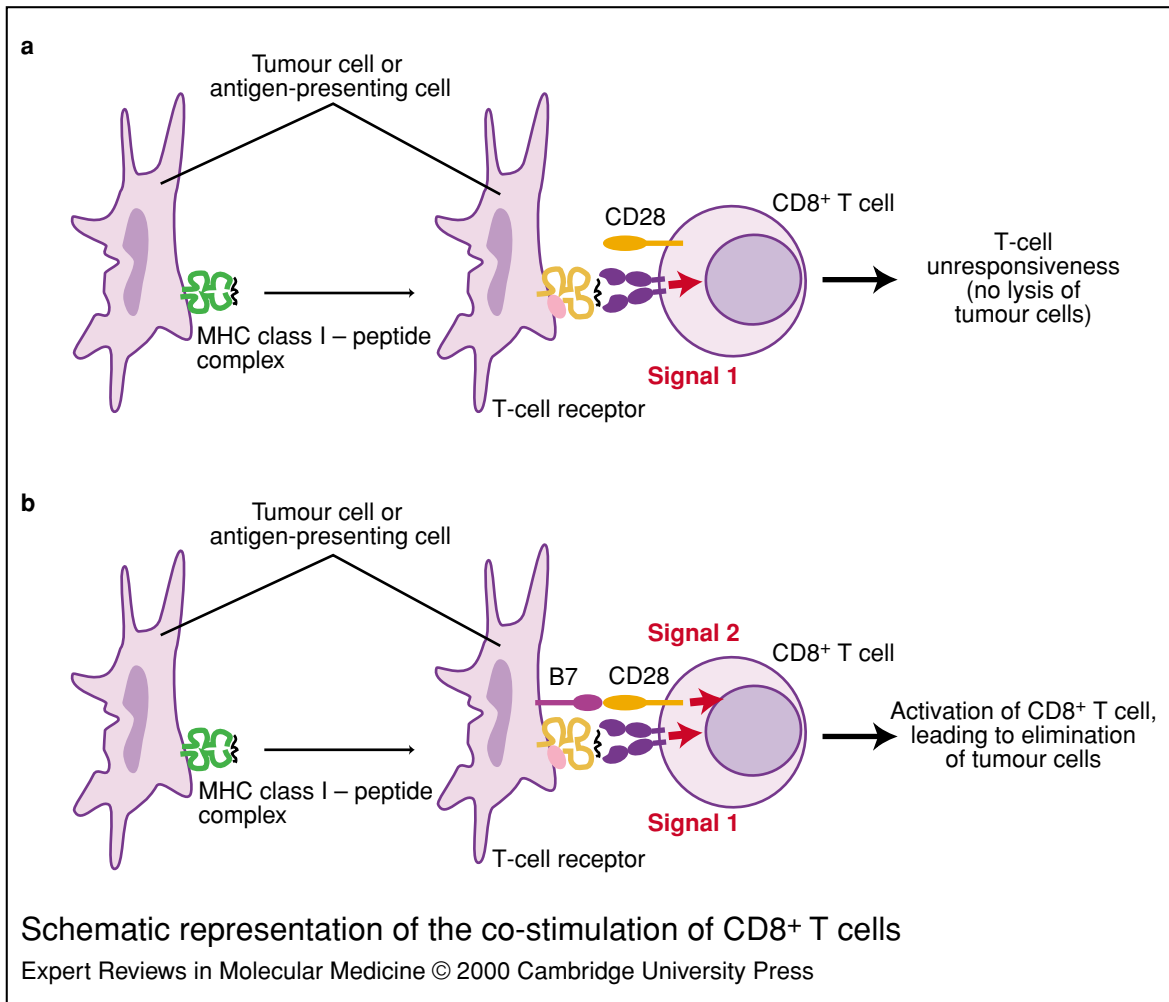
Co-stimulation of tumour-antigen-specific T cells

The activation of antigen-specific T cells, leading to cytokine production and proliferation, requires two separate signals (Ref. 95). The first signal is delivered to the T-cell receptor upon recognition of the peptide–MHC complex. The second signal can be delivered by CD28 molecules expressed on T cells after the engagement of the B7 co-stimulatory molecule expressed by activated APCs (Fig. 5). The importance of co-stimulation has been demonstrated by experiments that show that T cells do not respond when peptide–MHC or TCR recognition takes place in the absence of co-stimulatory molecules (Refs 96, 97). Other studies have shown that tumour cells can escape detection and subsequent elimination by T cells by the downregulation of co-stimulatory molecules on the tumour cell surface, or on dendritic cells presenting the tumour antigens (Ref. 98).

B7 co-stimulatory molecules

The B7 co-stimulatory molecules are homodimeric (i.e. express two identical, intertwined chains of the same protein) members of the immunoglobulin supergene family; they are found on the surface of cells that are capable of stimulating T-cell activation and proliferation. B7 molecules can bind to either CD28 or CTLA-4 on the surface of T cells. In the first instance, the ligation of CD28 (e.g. via B7 molecules or anti-CD28 monoclonal antibody) delivers an activating signal to the T cell, which induces the release of cytokines. After activation, T cells upregulate the expression of CTLA-4 on their cell surface, which also binds B7 molecules but delivers a negative signal, rendering the T cells less sensitive to further stimulation. The fate of T cells that respond to an antigenic stimulus depends on the balance between the stimulatory and inhibitory signals delivered to the T cell via these surface receptors. Likewise, T-cell activation can be enhanced by selectively stimulating CD28 or blocking CTLA-4 activity, and it can be inhibited by the reverse treatments (Ref. 99). The expression of B7 molecules on melanoma cells was found to elicit tumour rejection in mice; the anti-tumour response was mediated by CD8+ T cells (Ref. 100).

Using a mouse model, a mixture of vaccinia viruses expressing CEA and B7 molecules resulted in enhanced CEA-specific CTL responses and more-effective anti-tumour activity (Ref. 101). A clinical trial using an ALVAC virus expressing CEA and B7 molecules has been conducted on patients who have advanced CEA-expressing tumours. Eighteen patients were treated with monthly intramuscular injections; no significant side-effects were noted, including any evidence

Schematic representation of the co-stimulation of CD8+ T cells

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Figure 5. Schematic representation of the co-stimulation of CD8+ T cells. (a) The first activating signal ('signal 1') is delivered to the T-cell receptor by the complex formed between a major histocompatibility complex (MHC) molecule (MHC class I is shown here) and a peptide derived from an antigen [such as the tumour antigen carcinoembryonic antigen (CEA)]. (b) A second signal is delivered when B7 (CD80) molecules expressed on the (professional) antigen-presenting cell (APC; or tumour cell) bind to CD28 on the T cell. (a) If only the first signal is received, T-cell unresponsiveness occurs (i.e. T cells are not activated sufficiently for tumour cells to be lysed). (b) If both signals are received, the T cell becomes activated and tumours can be targeted and lysed by the CD8+ cytotoxic T cells. However, the expression of B7 molecules is often reduced on tumour cells, and this might be one of the ways by which these cells escape T-cell recognition **(fig005hka)**.

of autoimmune phenomena. Three patients experienced stabilisation of their disease, and this was associated with an increase in CEA-specific T-cell precursors, as measured by in vitro T-cell assays (H. Hörig and colleagues, in prep.). These findings support the use of B7 molecules as a vaccine adjuvant, and suggest that this approach will be safe and might be expected to elicit more-objective clinical responses in larger clinical trials involving patients whose disease is at an earlier stage.

CD40–CD40 ligand co-stimulatory molecules

Interactions between CD40 and CD40 ligand (also known as CD40L or CD154) represent another co-stimulatory system that has been widely studied (Fig. 6). The CD40 receptor is a 48-kDa protein, which is found on many cell types, especially APCs, such as B cells, dendritic cells, macrophages, monocytes, fibroblasts and endothelial cells. CD40L is a 39-kDa protein that belongs to the TNF family and is predominantly
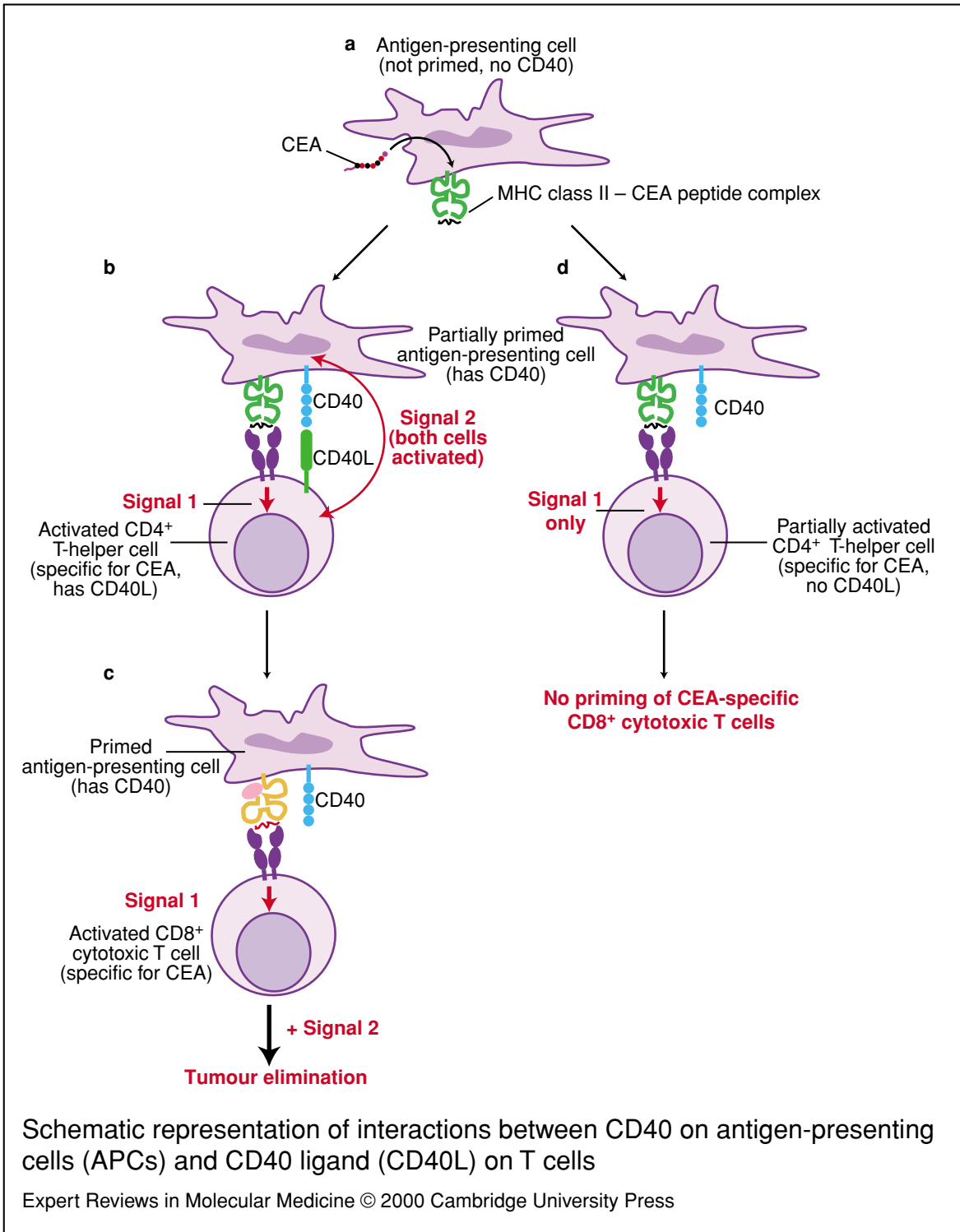
a Antigen-presenting cell
(not primed, no CD40)

CEA

MHC class II – CEA peptide complex

b

Partially primed
antigen-presenting cell
(has CD40)

CD40

**Signal 2
(both cells
activated)**

CD40L

Signal 1

Activated CD4+
T-helper cell
(specific for CEA,
has CD40L)

d

CD40

**Signal 1
only**

Partially activated
CD4+ T-helper cell
(specific for CEA,
no CD40L)

**No priming of CEA-specific
CD8+ cytotoxic T cells**

c

Primed
antigen-presenting cell
(has CD40)

CD40

Signal 1

Activated CD8+
cytotoxic T cell
(specific for CEA)

+ Signal 2

Tumour elimination

Schematic representation of interactions between CD40 on antigen-presenting
cells (APCs) and CD40 ligand (CD40L) on T cells

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**Figure 6. Schematic representation of interactions between CD40 on antigen-presenting cells (APCs)
and CD40 ligand (CD40L) on T cells** (*see next page for legend*) **(fig006hka).**

expressed on activated CD4+ T cells. Interactions
between CD40 and CD40L are important for
priming CTLs by CD4+ T cells, and might also

help induce humoral immunity (Ref. 102). To
test the role of CD40L in mediating anti-
tumour immunity, mouse tumour cells were

Figure 6. Schematic representation of interactions between CD40 on antigen-presenting cells (APCs) and CD40 ligand (CD40L) on T cells. (a) Carcinoembryonic antigen (CEA) protein (or peptide) is taken up from the extracellular space (e.g. from tumour cells) by APCs and CEA peptide fragments are presented by major histocompatibility complex (MHC) class II molecules on the APC. (b) T-cell receptors on a CEA-specific CD4+ T cell bind to the MHC–peptide complex, which delivers an initial activating signal (signal 1) to activate the T cells. (b) CD40 is expressed by partially primed APCs and binds to CD40L on activated CD4+ T-helper cells. This interaction delivers a second activating signal, which both fully activates the T cell and increases the priming of the APC. (c) This interaction between CD40 on the APC and CD40L on the T cell is critical for helping the APC to prime CEA-specific CD8+ cytotoxic T cells. (d) If CD40 on the APC does not bind to CD40L on the T-helper cell, the APC will not be able to prime CD8+ T cells to recognise CEA. T cells will not be able to attack CEA+ tumours and can continue to grow unchecked by the immune system. The role of B7 and CD28 is also very important in delivering secondary signals but has been omitted from this diagram for the sake of clarity (see Fig. 5). The roles and expression of CD40L in CD8+ T cells are less well understood than those in CD4+ T cells and have also been omitted here. Both CD40 and CD40L are candidates for inclusion in CEA-based cancer vaccines, with the aim to increase priming of APCs and CD8+ T cells for anti-CEA tumour-cell killing **(fig006hka)**.

transfected with the CD40L gene and inoculated subcutaneously into mice. This approach prompted tumour rejection, which was mediated by either natural killer cells or CD8+ CTLs (induced by CD4+ T cells), depending on the immunological phenotype of the mice (Ref. 103). To date, the potential benefits of increasing the expression of CD40L together with that of CEA to produce a novel tumour vaccine (i.e. vaccinia virus encoding CD40L and CEA) have not been experimentally evaluated.

Future challenges for CEA vaccine development
Animal models
The major goal of CEA-targeted immunotherapy is to be able to eradicate tumours but minimise damage to tissues that normally express CEA. As discussed, an increasing number of CEA vaccine strategies and host factors can influence the immune response to CEA. Much of our current understanding of CEA vaccine therapy stems from studies of immune-competent mice that have been transplanted with murine tumours that have been transfected with the human CEA gene. Although this model has been useful for comparing various treatment approaches, its direct biological relevance to humans is limited. These models are inadequate to address the issues of pre-existing tolerance (i.e. non-responsiveness) to self-tumour antigens in individuals who have cancer, or the potential for the development of autoimmunity after CEA vaccination. It would be valuable to assess these issues before these vaccines are applied in humans.

Transgenic mice that express the human CEA gene have been generated and represent

one potentially useful pre-clinical model for evaluating the full potential of CEA vaccine strategies. These mice express CEA in a spatio-temporal pattern that approximates that of normal CEA expression in humans, and can be used to determine the possible negative side-effects of immunotherapy, including autoimmunity (Ref. 104). In one study, CEA transgenic mice were subcutaneously transplanted with a mouse colon tumour expressing human CEA. The transgenic mice showed a faster tumour growth rate and were not able to develop anti-CEA antibodies, as compared with non-transgenic mice bearing a CEA-expressing tumour (Ref. 105). These studies suggest that tolerance against CEA can occur in an animal model and may represent a better model to assess strategies for vaccinating against CEA. In fact, a vaccinia virus expressing CEA could be used to break tolerance and improve therapeutic anti-tumour responses in CEA transgenic mice (Ref. 106).

Routes of administration
Animal models suggest that the route of administration may be an important issue for cancer vaccines. The comparative effectiveness of immunisation with a recombinant vaccinia virus expressing *LacZ* via the intravenous, subcutaneous or intradermal routes has been studied in mice. A significantly greater reduction in both the size and number of pulmonary metastasis occurred with intravenous immunisation (Ref. 107). The route of vaccination may also affect the type of immune response. DNA vaccinations given to mice through the intramuscular route resulted in the induction of different classes of T cells and antibodies against a model antigen

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than those induced by the same gene delivered through the intradermal route (Ref. 108). Recently, intrarectal immunisation using vaccinia virus expressing the HIV gp160 antigen enhanced systemic immunity in vaccinia-primed mice, whereas no augmentation was seen after intravenous immunisation (Ref. 109).

Several routes and methods of immunisation have been used for vaccination and these can have a significant effect on the type and strength of anti-tumour immunity. Vaccines can be administered systemically via subcutaneous, intradermal, intramuscular, intravenous, intratumoural, intralymphatic and intraperitoneal injection. Alternatively, they may be given through mucosal application, including intranasal, oral and rectal routes. However, to date, no randomised clinical trials have addressed the issue of which routes are better for the administration of cancer vaccines.

Prime and boost strategies

The generation of multiple vectors for vaccination, and the development of neutralising antibodies that prevent repetitive exposure to a single vector, has led to the use of prime and boost strategies. Such protocols prime the immune response with one vector expressing an antigen, and then boost with a different vector expressing the same antigen. Heterologous boosting of mice using first vaccinia virus and then ALVAC virus expressing CEA improved tumour responses and CTL activity against CEA, compared to those produced by vaccination with either virus alone (Ref. 110). Clinical trials are beginning to suggest similar results. In one study, vaccinia and ALVAC viruses expressing CEA were administered alternately; the highest levels of CEA-specific precursor T cells were generated by the group of patients who were primed with vaccinia-CEA vaccine before being given ALVAC-CEA vaccine (Ref. 94). Similar results were obtained in a trial that primed patients with vaccinia virus expressing CEA and then boosted them with CEA peptides (Ref. 111).

Research in progress and unanswered questions

CEA was one of the first tumour antigens to be isolated from cancer patients and is expressed on many different types of tumours. The molecular biology of CEA has been extensively studied and the gene that encodes it has been cloned. Less is known about the immune responses to CEA in cancer patients, although studies have suggested the generation of both antibodies and T-cell responses are possible. The identification of T-cell epitopes within CEA led to the development of numerous recombinant and synthetic vaccine strategies for immunisation. These strategies include the use of CEA peptides, recombinant CEA protein, recombinant bacteria and viruses expressing CEA, CEA-pulsed dendritic cells and anti-CEA monoclonal antibodies. A better understanding of how immune responses are generated has resulted in the addition of cytokines and co-stimulatory molecules as adjuvants to CEA-directed vaccines. These strategies have improved the induction of immune responses to CEA in animal models and, in some cases, in patients treated in early-phase clinical trials. Current problems include the lack of an adequate animal model and limited knowledge about the optimal routes of administration and dosing schedule for vaccination. The early clinical trials suggest that vaccination with CEA vaccines is safe, producing few side-effects, and can lead to CEA-specific immunity. Additional research to define the best approach to vaccination and intervention at earlier stages of disease will further improve the effectiveness of vaccine therapy. CEA remains a useful target for the development of vaccines for the treatment and prevention of cancer.

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Features associated with this article

Figures

Figure 1. Schematic representation of the human carcinoembryonic antigen (CEA) gene and protein (fig001hka).

Figure 2. Mechanisms that target and destroy tumour cells following the binding of anti-carcinoembryonic antigen (CEA) monoclonal antibodies to CEA-expressing tumour cells (fig002hka).

Figure 3. Schematic diagram showing how carcinoembryonic antigen (CEA) induces anti-tumour responses mediated by CD4^{+} and CD8^{+} T cells (fig003hka).

Figure 4. Schematic representation of how the modification of peptides can influence affinity binding to major histocompatibility complex (MHC) class I molecules or the T-cell receptor (TCR) (fig004hka).

Figure 5. Schematic representation of the co-stimulation of CD8^{+} T cells (fig005hka).

Figure 6. Schematic representation of interactions between CD40 on antigen-presenting cells (APCs) and CD40 ligand (CD40L) on T cells (fig006hka).

Table

Table 1. Carcinoembryonic antigen (CEA) peptides that are recognised by human CD8^{+} T cells (tab001hka).

Further reading, resources and contacts

The American Association for Cancer Research (AACR) is a scientific society of cancer researchers, which facilitates the communication and dissemination of knowledge among scientists and others concerned with the cancer problem.
　　http://www.aacr.org/

The American Cancer Society (ACS) is a US community-based voluntary health organisation that is dedicated to eliminating cancer as a major health problem through research, education, advocacy and service.
　　http://www.cancer.org/

The US Cancer Research Institute (CRI) is a source of funding for cancer and immunology research.
　　http://www.cancerresearch.org/

The National Cancer Institute (NCI) website provides information on cancer, funding for scientists and partnerships.
　　http://www.nci.nih.gov

The ClinicalTrials.gov website (a service provided by the National Institutes of Health, and developed by the National Library of Medicine) provides useful information about clinical trials.
　　http://clinicaltrials.gov/ct/gui

The US National Center for Biotechnology Information (NCBI) is a national resource of biomedical information – all for the better understanding of molecular processes affecting human health and disease.
　　http://www.ncbi.nlm.nih.gov/

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