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The next wave of recombinant and synthetic anticancer vaccines

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Abstract

The identification of tumor-associated antigens (TAA) recognized by T lymphocytes makes the development of antigen-specific synthetic and recombinant vaccines possible. The expression of TAA within a recombinant vector increases control over the kinetics and quantity, the molecular form, and the subcellular location of the immunogen delivered. The next generation of antitumor vaccines employs cytokines and costimulatory molecules expressed in concert with TAA that are capable of augmenting the activation and proliferation of antitumor immune responses. The ultimate goal of these new strategies, the treatment of established cancer, is now being realized in animal models.

Keywords

tumor-associated antigen; recombinant vaccines; immunotherapy; recombinant fowlpox; recombinant adenovirus

Elements of the cellular immune system clearly play a role in antitumor immunity.¹⁻⁴ The recent cloning of new TAA recognized by T lymphocytes allows for the development of recombinant and synthetic anticancer vaccines. Combining new techniques in molecular biology, virology and synthetic protein chemistry, specific vaccines can be developed that increase the control the immunotherapist has over the quantity and kinetics of TAA expression, the intracellular compartment into which the TAA are expressed and what cell types or tissues are used to express TAA *in vivo*. In addition to the classic concept of viral vaccination in which individuals are immunized against antigens prior to natural challenge with the pathogen, therapeutic cancer vaccination may also be used to induce or enhance antitumor immune reactions in patients who already have cancer. This review will discuss examples of current recombinant and synthetic vaccine strategies designed to enhance or elicit antitumor responses.

Tumor associated antigens recognized by T lymphocytes have been identified

Recently, a number of molecules have been described that represent antigens that serve as T-cell targets.⁵⁻⁸ These tumor-associated antigens are described in greater detail in previous reviews in this Seminar. They can be grouped into several different categories. One group of potential TAA consists of viral gene products expressed by virally induced tumors.⁹ Viruses associated with the development of human cancers include hepatitis B viruses in hepatocellular carcinomas; human papilloma viruses (HPV) in anogenital cancers; Epstein-Barr virus (EBV) in Hodgkin's and Burkitt's lymphomas as well as nasopharyngeal carcinomas; and human T-cell leukemia/lymphoma viruses in adult T-cell leukemia/lymphoma.¹⁰⁻¹³ Late membrane protein gene products from EBV have been shown to be important for cell growth control and are found expressed within lymphoid tumors including Hodgkin's disease and nasopharyngeal carcinomas.¹⁴ Human papilloma virus E6 and E7 transforming proteins, expressed within cervical tumors, may also be candidate immunogens.^{15,16}

A second group of antigens identified initially through the use of monoclonal antibodies or from T cells obtained from tumor tissue samples have turned out to be predominantly non-mutated proteins. Although antigens identified by antibodies have not been shown to be entirely tumor specific, they are routinely utilized as an aid in the diagnosis of disease and post treatment monitoring of the response to therapy since they may be overexpressed in the tumor bearing patient. Carcinoembryonic antigen (CEA), one of the most widely studied TAA, is a large molecule found on the surface of gastrointestinal, breast and lung carcinomas.¹⁷⁻¹⁹ MUC-1 human breast epithelial antigen and gp100 human melanoma differentiation antigen represent other examples of potential targets initially recognized by monoclonal antibodies. All have recently been shown to be recognized by T lymphocytes as well.²⁰⁻²² Most of the genes encoding TAA recognized by anti-tumor T lymphocytes, mainly in melanoma, have thus far been non-mutated, differentiation antigens also expressed in normal melanocytes. These antigens, described in several reviews, include gp100, MART-1/Melan A, TRP-1, and tyrosinase.⁵⁻⁸ MAGE, BAGE and GAGE are also non-mutated proteins found in some melanomas and several carcinomas but not in most normal tissues with the exception of testes.

Recent studies with antitumor T lymphocytes have also identified epitopes derived from point mutated products, novel peptides resulting from translation of frameshifts and introns²³⁻²⁶ (Wang, R. *et al*, in press). Antimelanoma T lymphocytes have also identified a point mutation of the β -catenin protein, known to be associated with cell adhesion molecules (Robbins *et al*, unpublished results).

Several groups have taken an alternative approach of eliciting T lymphocytes against proteins involved in the process of malignant transformation such as oncogenes or mutated suppressor gene products.²⁷⁻³² T cells recognizing peptides derived from BCR-ab1 translocation break points and mutations of ras p21 oncogene or p53 tumor suppressor molecule have now been defined, although mutations vary between patients and prescreening may be required. Furthermore, it will be important to determine whether these mutations are normally processed and presented in the context of the patients' own HLA molecules before such epitopes are used in cancer vaccination.

Antigen processing: where cell mediated immune responses begin

T lymphocytes recognize antigen not as native, folded protein as antibodies do, but as fragments of proteins complexed with MHC molecules.³³⁻³⁶ The processing of antigens for presentation by MHC class I to CD8⁺ T lymphocytes differs dramatically from the processing of antigens for presentation of class II MHC molecules to CD4⁺ T lymphocytes. In the class I pathway, intracellular antigenic proteins are cleaved in the cytosol into short 8–10 amino acid long peptides, possibly by the activity of cytosolic protease activity residing within the large multicatalytic proteasome.³⁷ Peptides are generally transported into the endoplasmic reticulum (ER) by a protein heterodimer called TAP (transporter associated with antigen presentation). The peptides then associate with class I α chain and β 2 microglobulin in a trimolecular complex with is then transported to the cell surface.³⁸

For MHC class II presentation to CD4⁺ T cells, antigen enters the endosomal and lysosomal compartments of antigen presenting cells (APC) where they are degraded by acid proteases into peptide fragments 12–24 amino acids in length.^{33,34,36} Exogenous proteins are either phagocytosed or endocytosed by the APC whereas endogenous proteins, usually membrane bound, are thought to simply internalize from the cell surface. Although cytosolic proteins have been described to enter the class II pathway, it is believed that the majority of the cytosolic proteins do not bind class II MHC molecules because they are protected in the ER by the invariant chain CLIP region.³³ For both MHC class I and class II peptides, allele-specific

epitope forecasts have been devised because of the existence of motifs for the amino acid residues involved in the binding of the peptide to the MHC molecule.^{36,39,40}

Targeting antigen to the class I and class II pathways

Specific sequences exist that can target different subcellular organelles. The addition of an ER signal sequence to the 5' end of a peptide can bypass the TAP pathway and target the peptide for co-translational insertion into the ER.⁴¹⁻⁴³ The addition of the E3/19k leader sequence of adenovirus preceding the minimal determinant of a murine tumor antigen (P815A) expressed within recombinant vaccinia virus enhanced the immunogenicity of the minimal determinant, presumably by increasing the concentration of peptide complexed to MHC class I in the ER lumen.⁴⁴

The addition of a COOH-terminal domain of lysosome membrane proteins, either LAMP-1, LAMP-2 or lysosomal acid phosphatase, to heterologous proteins has been shown to localize the resulting fusion products to the lysosomal compartment resulting in protein degradation and enhanced presentation to MHC class II-restricted T cells.^{45,46} These tubulo-vesicular lysosomes appear to be involved in the endocytic, phagocytic and biosynthetic pathways in which antigens may be processed for presentation by MHC class II molecules and subsequent transport to the cell surface.⁴⁷

Examples of synthetic and recombinant vaccine approaches

A number of potential strategies to induce active immunotherapy responses in cancer patients are currently being explored (Table 1). Many studies have utilized physically altered whole tumor cells, tumor cell extracts, or viral oncolysates as vaccines. Other whole tumor approaches have included gene modified tumor cells that express cytokines or co-stimulatory molecules.⁴⁸ These kinds of approaches take advantage of the multitude of antigens that are expressed by the tumor cell, obviating the need to know the identity of the tumor antigens. However, whole tumor cells may express low quantities of the immunogenic antigens, may process antigens poorly, may express suppressive factors or may lack adhesion or costimulatory molecules necessary to mount an efficient immune response. Synthetic and recombinant immunization approaches avoid these potential problems.

Synthetic peptides

As the details of antigen presentation have been elucidated, there has been an increasing interest in the use of minimal antigenic determinants as immunogen. Different immunization protocols utilized to induce immune responses include peptides coated onto the surface of splenocytes or dendritic cells, formulated with immune stimulating complexes (ISCOM) entrapped in lysosomes, osmotically loaded into splenocytes or covalently linked to a lipophilic compound or to a T helper epitope.⁴⁹⁻⁵⁷ Strategies of modification of peptides to either substitute amino acids to augment binding affinity to MHC molecules or to eliminate deleterious amino acids are also being pursued.^{58,59} Approaches for identifying immunogenic peptides and augmenting their immunogenicity are described in greater detail in the previous review.

Recombinant vaccinia virus (rVV)

Vaccinia virus was demonstrated to be a safe and effective immunogen in the successful, world-wide campaign to eliminate smallpox.⁶⁰ A member of the DNA-containing poxvirus family, VV has a large genome of 185,000 bp which encodes over 100 genes. Approximately 25,000 bp of foreign DNA can be stably inserted into a number of sites within the VV genome by homologous recombination without affecting replication or packaging of the virus. The foreign genes are accurately transcribed and translated within host cell cytoplasm into functional protein products.

Recombinant vaccinia viruses (rVV) developed in several tumor model systems have shown some promise in the induction of potent cellular and humoral immune responses correlating with protection after subsequent challenge with tumor.⁶¹⁻⁶⁷ In some cases, active immunization alone resulted in prolonged survival or treatment of mice bearing small doses of established tumor.^{61,63,65} A peptide from CEA, CAP-1, was capable of eliciting HLA A-2 restricted CTL *in vitro* from precursors present in PBL from cancer patients immunized with rVV-CEA.²⁰ As an alternative to active immunization alone, rVV has also elicited antigen-specific CTL that could be adoptively transferred to therapeutically treat mice bearing tumor.^{44,68}

The kinetics and quantity of recombinant antigen production by rVV appears to affect the immune response observed. The expression of heterologous genes introduced into the viral genome is dependent on a VV promoter being located 5' to the inserted gene in the recombinant virus.^{69,70} Following viral entry into the cell cytoplasm, the VV genes are regulated in a temporal manner.⁷¹ Immediately following infection, the class of early genes are transcribed and packaged into progeny virions. The early immediate gene products insure that the RNA undergo proper post-transcriptional modifications, including capping, methylation, and poly (A) adenylation. Late genes are expressed following the onset of DNA replication when the host protein synthesis has been inhibited by the VV. A comparison of rVV using different promoters revealed that antigens expressed by early promoters primed both B and T-cell responses to HA antigen of influenza virus whereas late promoters only elicited humoral immunity.⁷¹ Totally synthetic early/late promoters have also been developed that express 40-fold more antigen than the p-7.5 early promoter, much of it expressed late in viral replication (Chakrabati, S., Sissler, J. and Moss, B., unpublished data). Unpublished work in our own laboratory suggests that a construct utilizing the synthetic early/late promoter may mediate more potent antitumor effects in a pulmonary metastasis model than a number of early or late promoters derived from VV p-7.5 (Bronte *et al.*) The kinetics and quantity of antigen expression by the rVV appear to be important factors in the induction of optimal antitumor immunity.

Since individuals born before 1972 received previous smallpox vaccination, the presence of long-lasting immune memory against vaccinia proteins may result in rapid elimination of the rVV, potentially reducing immune responses against the tumor antigen expressed by the rVV.⁷²⁻⁷⁴ However, despite the anamnestic responses observed in humans, rVV has been shown to prime B and T-cell responses to inserted gene products, which could be boosted by immunization with purified recombinant proteins.^{72,73} A second potential disadvantage to the use of rVV is that it is a live replication competent virus which has potential to result in disseminated viremia, especially in immunocompromized individuals.^{73,75} For these reasons, a variety of nonreplicating recombinant viral and non-viral systems have been developed.⁷⁶

Nonreplicating poxviruses

Fowlpox virus, an Avipox virus, causes a slow spreading pox disease in chickens, but does not productively replicate in mammalian cells.⁷⁷ However, in mammalian cells, rFPV maintain expression of foreign genes which make them attractive candidates for a safe viral vaccine. Protective immune responses were generated by rFPV against rabies glycoprotein and more recently in an experimental tumor model system.⁷⁸ rFPV could not only protect mice against a tumor challenge, but could also treat established tumor.⁷⁹ Importantly, prior immunization with VV did not abrogate immune responses elicited by rFPV.⁷⁹ Another Avipox virus, canarypox virus (CPV), can also express inserted genes in the absence of viral replication in mammalian cells.⁸⁰ rCPV has been reported to induce antibody and CTL immunity against a variety of antigens, including rabies virus glycoprotein.⁸¹⁻⁸³

Modified Virus Ankara (MVA) was derived from vaccinia virus by repeated serial passages in chick embryo fibroblast cells. MVA has at least six deletions resulting in the loss of ability to replicate in mammalian cells.⁸⁴ MVA has an advantage over the avipox viruses in that late as well as early expression of virus genes is unaffected because the block in viral replication occurs at a step in viral assembly.⁸⁵ Preliminary studies in our laboratory have shown that recombinant MVA vectors have therapeutic utility in a mouse tumor model (Carroll *et al*, submitted for publication).

Recombinant adenovirus (rAd)

Military recruits in the USA and Canada for the last 30 years have ingested adenovirus to successfully vaccinate against adult respiratory disease without deleterious side effects.^{86,87} rAd has been utilized as a gene delivery vector for therapy of patients with cystic fibrosis, atherosclerotic disease and anti-1-alpha-trypsin deficiency among others. Insertion of heterologous sequences has generally been accomplished within the E1, E3 and E4 regions using respective endogenous early promoters or the potent major late promoter. Since the E1 region is responsible for the regulation of a number of adenoviral genes, including those responsible for DNA replication, substitution of this region renders the virus replication incompetent and less likely to induce immune responses against adenoviral proteins. Unlike the poxviruses described above, rAd can use host cell polymerases to transcribe its genes thus opening up the possibility for the use of tissue specific promoters. In work done in our laboratory, immunization of rAd expressing β -gal using intravenous, intramuscular, and intranasal routes have been successful at generating specific, lytic CTL, which could be transferred adoptively to therapeutically treat tumor bearing mice. Furthermore, immunization of mice bearing established tumor with high titers of an E1/E4 deleted rAd expressing a model TAA were able to actively reduce the number of pulmonary tumor nodules (Chen *et al*, in press, J Immunol). Thus, nonreplicating adenoviruses may also have utility in the management of cancer.

Recombinant retroviruses

Retroviruses represent another nonreplicating vector for the delivery of foreign proteins to the class I MHC antigen processing pathway. Direct intramuscular injection of retroviruses encoding heterologous HIV-1 proteins generated antigen specific T_{CD8+} in mice, macaques and baboons.⁸⁸

Recombinant bacteria

Bacillus Calmette-Guerin, BCG, an attenuated vaccine strain of *Mycobacterium bovis*, has been engineered for use as an antigen delivery vehicle.⁸⁹ BCG has been administered world-wide as a vaccine against tuberculosis since 1948 with few complications. Components of the cell wall of BCG have powerful adjuvant activity. Recent advances in molecular techniques have resulted in the development of improved mycobacterial expression vectors that replicate in both *E. coli* and mycobacteria, thus avoiding the difficulties with manipulations of mycobacteria which have long doubling times compared to *E. coli*.^{90,91} Long lasting antibody, T-helper and cytotoxic T-immune response were induced in mice following immunization with BCG containing the gene for β -gal.⁸⁹ Other antigens, including leishmania, Lyme disease OspA protein, and *Borrelia burgdorferi* outer surface protein, delivered by rBCG elicited protective immunity from challenge with the respective pathogen.⁹²⁻⁹⁴

Listeria monocytogenes is a gram positive, facultative intracellular bacteria that may also be useful in recombinant vaccine design. *Listeria monocytogenes* enters host cells and is taken up by the phagosomes where it then escapes into the cytoplasm by disrupting the phagosomal membrane primarily using hemolysin.⁹⁵ The hemolysin-dependent bacterial escape into the cytoplasm has been demonstrated to be critical for class I presentation of antigen.⁹⁶ This unique

access of these bacteria into the host cell allows access into both the class I and class II MHC pathways. *Listeria monocytogenes* expressing β -gal was able to elicit specific cellular and humoral immune responses specifically against β -gal.⁹⁷ Recently, *L. monocytogenes* expressing a model tumor antigen was demonstrated to protect mice from lethal tumor challenge as well as cause regression and induces regression of established tumors.⁹⁸

DNA-based immunization

DNA-based vaccination is accomplished by the expression of plasmid DNA encoding the antigen of interest preceded by a eukaryotic promoter. Skeletal muscles have demonstrated the ability to take up and express DNA without a specific delivery system.^{99,100} Alternatively, DNA coated gold particles can be physically delivered into living cells using a hand-held helium-powered device or 'gene gun'.¹⁰¹⁻¹⁰⁴ The technique can be used to deliver DNA to liver, skin and muscle with as much as 20% of the cells expressing antigen within the field of immunization.¹⁰⁵ The plasmid exists episomally and generally does not integrate into the genome. Both DNA-based approaches have been shown to successfully induce both cellular and humoral immunity in many antigen systems.^{103,104,106-109} Intramuscular immunization with plasmid constructs encoding full-length cDNA for CEA or HIV-1 vev and GP160 proteins have been shown to protect mice from challenge with syngeneic tumors expressing these model antigens.^{110,111} Our own work indicates that the 'gene gun' delivery of plasmid DNA could elicit specific lytic T lymphocytes effective upon adoptive transfer to mice bearing established metastases (Irvine *et al*, in press). Most interestingly, the therapeutic effects of 'gene gun' delivery of DNA could be greatly enhanced when followed by T-cell activating or proliferating cytokines delivered systemically (Irvine *et al*, in press, J Immunol). DNA-based vaccines offer several advantages over recombinant viruses. Purified DNA is relatively safe and can be easily and rapidly purified compared to viruses. The use of plasmid DNA would also eliminate the problems of anamnestic responses that lead to rapid clearance of viral constructs. Reduced immune responses against the vector may also increase the efficacy of multiple boosts.

The vectors developed for the delivery of recombinant foreign gene products can be further characterized by their dependence on the host for transcriptional and translational regulation (Figure 1). Recombinant poxviruses transcribe the inserted genes using their own viral polymerases but rely on the host for translational events. Bacterial vectors, on the other hand, are self-sufficient and produce their own proteins, although they are not capable of performing eukaryotic post-transcriptional modifications. In contrast, rAd and intramuscular or 'gene gun' administration of plasmid DNA contribute solely DNA and rely on host proteins to initiate transcription and translation. Retroviruses contain their own RNA and provide reverse transcriptase but also utilize host cell machinery for translation of inserted proteins. Vectors that rely on host cell translation including poxviruses, adenoviruses, retroviruses and plasmid DNA as examples, may have an advantage because they can utilize normal intracellular trafficking pathways to target antigens to subcellular compartments (Table 1). In addition, the host cell translational machinery can also direct the synthesis of integral membrane glycoproteins such as costimulatory molecules.

Additional approaches for augmenting the antitumor immune response

Tumor specific T lymphocytes found within the tumor mass are only observed following an in-vitro stimulation with tumor; suggesting either a lack of activation or a tolerance of the normal immune response occurs against the TAA. Many of the TAA identified thus far are tissue specific or self antigens, especially in the case of melanoma; the immune system may thus be tolerant to them. It is now known that a second signal in addition to that provided by engagement of the TCR is important for T lymphocyte activation.¹¹⁵ Costimulatory molecules found on APC, such as B7-1 B7-2, engage CD28 or CTLA4 on T cells providing the necessary second signal. T-cell receptors binding to Ag/MHC complexes alone without costimulatory

molecules can result in T-cell anergy. Also other aspects of the tumor microenvironment may be inefficient, resulting in the inactivation of the tumor specific T cells. The expression of tumor antigen may be too low to stimulate immunity. In addition, without activation of T-helper lymphocytes or other immune cells, the cytokine micromilieu may not include enough T cell proliferating or activating factors to promote lymphocyte activation and tumor destruction.

A number of studies have demonstrated that antitumor effects can be augmented if the tumor cells are transduced with either of two costimulatory molecules, B7-1 and B7-2.¹¹³⁻¹¹⁶ Immunization with murine carcinoma cells infected with an rVV containing either B7-1 or B7-2 resulted in delayed growth of a subsequent challenge with wild type tumor, presumably by supplying a second signal to activate T-cell immunity.¹¹⁷ Immunization with admixtures of an rVV encoding B7-1 and an rVV encoding a TAA resulted in enhanced protection from the growth of tumor cells compared to the administration of either rVV-B7-1 or rVV-TAA alone.¹¹⁸ rVV encoding both TAA, β -gal and either B7-1 or B7-2 administered in an active immunotherapy setting significantly enhanced the ability of rVV containing β -gal alone to reduce pulmonary metastases and prolonged survival of these mice (Chamberlain *et al* submitted for publication). Together, these data suggest that second signals from costimulatory molecules can enhance tumor immunity.

rhIL-2 encoded within rVV expressing TAA β -gal or administered exogenously with rVV- β -gal significantly enhanced reduction of pulmonary metastases compared to the partial responses observed with rVV alone.¹¹⁹ These responses correlated with an increase in primary CTL activity suggesting that IL-2 may be functioning to increase the precursor frequency of CD8+ anti- β -gal T lymphocytes.¹¹⁹ Work done in our laboratory has shown that IL-10 or IL-12 each administered systemically as adjuvants to mice immunized with rVV expressing β -gal each significantly augmented reduction in the number of β -gal expressing tumors compared to rVV or cytokine alone (Rao *et al* submitted). IL-10, reported to activate Th2-like responses, is thought here to be directly activating cytolytic T-cell activity or have direct effects prolonging viral persistence.¹²⁰ IL-12 has been reported to augment the helper T-cell response to a Th1-like response, supporting and activating cellular immunity.¹²¹ Exogenous IL-12 can also cooperate *in vivo* with B7-1 to increase therapeutic activities observed with rVV encoding B7-1 and TAA β -gal (Rao *et al*, submitted), rVV vectors elaborating multiple TAAs, costimulatory and cytokine molecules are now being developed.

This review described examples of different approaches to anticancer vaccination. It is difficult to compare these different strategies in murine model systems because the responses to each of these vectors may differ dramatically from mouse to human, depending on a number of factors. These include tolerance toward the TAA, the presence of preexisting immune responses against the vectors themselves, the tropism of the viral or nonviral carrier for the host and species differences in the effects of cytokines and costimulatory molecules. The use of recombinant and synthetic vaccines has thus far demonstrated encouraging preclinical results for the treatment of established cancer in animal model systems. Our aim is that this will soon be translated into the clinical setting.

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References

1. Greenberg PD, Cheever MA, Fefer A. H-2 restriction of adoptive immunotherapy of advanced tumors. *J Immunol* 1981;126:2100–2103. [PubMed: 6453154]

2. Rosenberg SA, Packard BS, Aebersold PM, Solomon D, Topalian SL, Toy ST, Simon P, Lotze MT, Yang JC, Seipp CA, Simpson C, Carter C, Bock S, Schwartzentruber D, Wei JP, White DE. Use of tumor infiltrating lymphocytes and interleukin-2 in the immunotherapy of patients with metastatic melanoma. Preliminary report. *N Engl J Med* 1988;319:1676–1680. [PubMed: 3264384]
3. Greenberg PD. Adoptive T cell therapy of tumors: mechanisms operative in the recognition and elimination of tumor cells. *Adv Immunol* 1991;49:281–355. [PubMed: 1853786]
4. Hellstrom KE, Hellstrom I. Cellular immunity against tumors. *Adv Cancer Res* 1969;12:167–223. [PubMed: 4898911]
5. Houghton AN. Cancer antigens: immune recognition of self and altered self. *J Exp Med* 1994;180:1–4. [PubMed: 8006576]
6. Pardoll DM. Tumour antigens. A new look for the 1990s. *Nature* 1994;369:357. [PubMed: 8018207]
7. Boon T, Gajewski TF, Coulie PG. From defined human tumor antigens to effective immunization? *Immunol Today* 1995;16:334–336. [PubMed: 7576068]
8. Rosenberg SA. The development of new cancer therapies based on the molecular identification of cancer regression antigens. *Cancer J* 1995;1:89–101.
9. Chow VTK. Cancers and viruses. *Ann Acad Med* 1993;22:163–168.
10. Durst M, Gissman L, Ikenberg H, zur Hausen H. A papillomavirus DNA from a cervical carcinoma and its prevalence in cancer biopsy samples from different geographic regions. *Proc Natl Acad Sci USA* 1983;80:3812–3815. [PubMed: 6304740]
11. Pallesen G, Hamilton-Dutoit SJ, Rowe M, Young LS. Expression of Epstein-Barr virus latent gene products in tumour cells of Hodgkin's disease. *Lancet* 1991;377:320–322. [PubMed: 1671232]
12. Young LS, Dawson CW, Clark D, Rupani H, Busson P, Tursz T, Johnson A, Rickinson AB. Epstein-Barr virus gene expression in nasopharyngeal carcinoma. *J Gen Virol* 1988;69:1051–1065. [PubMed: 2836550]
13. Young LS, Yao QY, Rooney CM, Sculley TB, Moss DJ, Rupani H, Laux G, Bornkamm GW, Rickinson AJ. New type B isolates of Epstein-Barr virus from Burkitt's lymphoma and from normal individuals in endemic areas. *J Gen Virol* 1987;68:2853–2862. [PubMed: 2824665]
14. Moss DJ, Burrows SR, Suhrbier A, Khanna R. Potential antigenic targets on Epstein-Barr virus-associated tumours and the host response. *Ciba Found Symp* 1994;187:4–13. [PubMed: 7796675]
15. Chen L, Mizuno MT, Singhal MC, Hu SL, Galloway DA, Hellstrom I, Hellstrom KE. Induction of cytotoxic T lymphocytes specific for a syngeneic tumor expressing the E6 oncoprotein of human papillomavirus type 16. *J Immunol* 1992;148:2617–2621. [PubMed: 1313847]
16. Kast WM, Brandt RM, Drijfhout JW, Melief CJ. Human leukocyte antigen-A2.1 restricted candidate cytotoxic T lymphocyte epitopes of human papillomavirus type 16 E6 and E7 proteins identified by using the processing-defective human cell line T2. *J Immunother* 1993;14:115–120.
17. Gold P, Freedman SO. Specific carcinoembryonic antigens of the human digestive system. *J Exp Med* 1965;122:467. [PubMed: 4953873]
18. Muraro R, Wunderlich D, Thor A, Lundy J, et al. Definition by monoclonal antibodies of a repertoire of epitopes in human colon carcinomas versus normal adult tissues. *Cancer Res* 1985;45:5769–5780. [PubMed: 2413997]
19. Steward AM, Nixon D, Zamcheck N, Aisenberg A. Carcinoembryonic antigen in breast cancer patients: serum levels and disease progress. *Cancer* 1974;33:1246. [PubMed: 4823478]
20. Tsang KY, Zaremba S, Nieroda CA, Zhu MZ, Hamilton JM, Schlom J. Generation of human cytotoxic T cells specific for human carcinoembryonic antigen epitopes from patients immunized with recombinant vaccinia-CEA vaccine. *J Natl Cancer Inst* 1995;87:982–990. [PubMed: 7629885]
21. Acres RB, Hareuveni M, Balloul J-M, Kieny M-P. Vaccinia virus MUC1 immunization of mice: Immune response and protection against the growth of murine tumors bearing the MUC1 antigen. *J Immunother* 1993;14:136–143.
22. Bakker AB, Schreurs MW, de Boer AJ, Kawakami Y, Rosenberg SA, Adema GJ, Figdor CG. Melanocyte lineage-specific antigen gp100 is recognized by melanoma-derived tumor-infiltrating lymphocytes. *J Exp Med* 1994;179:1005–1009. [PubMed: 8113668]
23. Monach PA, Meredith SC, Siegel CT, Schreiber H. A unique tumor antigen produced by a single amino acid substitution. *Immunity* 1995;2:45–59. [PubMed: 7600302]

24. Wang RF, Robbins PF, Kawakami Y, Kang XQ, Rosenberg SA. Identification of a gene encoding a melanoma tumor antigen recognized by HLA-A31-restricted tumor-infiltrating lymphocytes. *J Exp Med* 1995;181:799–804. [PubMed: 7836932]
25. Townsend A, Ohlen C, Rogers M, Edwards J, Mukherjee S, Bastin J. Source of unique tumour antigens. *Nature* 1994;371:662. [PubMed: 7935811]
26. Coulie PG, Lehmann F, Lethe B, Herman J, Lurquin C, Andraniss M, Boon T. A mutated intron sequence codes for an antigenic peptide recognized by cytolytic T lymphocytes on a human melanoma. *Proc Natl Acad Sci USA* 1995;92:7976–7980. [PubMed: 7644523]
27. Disis ML, Smith JW, Murphy AE, Chen W, Cheever MA. In vitro generation of human cytolytic T-cells specific for peptides derived from the HER-2/neu protooncogene protein. *Cancer Res* 1994;54:1071–1076. [PubMed: 7508819]
28. Peace DJ, Smith JW, Chen W, You SG, Cosand WL, Blake J, Cheever MA. Lysis of ras oncogene-transformed cells by specific cytotoxic T lymphocytes elicited by primary in vitro immunization with mutated ras peptide. *J Exp Med* 1994;179:473–479. [PubMed: 8294861]
29. Melief CJ, Kast WM. Potential immunogenicity of oncogene and tumor suppressor gene products. *Curr Opin Immunol* 1993;5:709–713. [PubMed: 8240732]
30. Ciernik IF, Berzofsky JA, Carbone DP. Mutant oncopeptide immunization induces CTL specifically lysing tumor cells endogenously expressing the corresponding intact mutant p53. *Hybridoma* 1995;14:139–142. [PubMed: 7590770]
31. Yanuck M, Carbone DP, Pendleton CD, Tsukui T, Winter SF, Minna JD, Berzofsky JA. A mutant p53 tumor suppressor protein is a target for peptide-induced CD8+ cytotoxic T-cells. *Cancer Res* 1993;53:3257–3261. [PubMed: 7686815]
32. Cheever MA, Chen W, Disis ML, Takahashi M, Peace DJ. T-cell immunity to oncogenic proteins including mutated ras and chimeric bcr-abl. *Ann NY Acad Sci* 1993;690:101–112. [PubMed: 8103658]
33. Germain RN. MHC-dependent antigen processing and peptide presentation: providing ligands for T lymphocyte activation. *Cell* 1994;76:287–299. [PubMed: 8293464]
34. Accolla RS, Adorini L, Sartoris S, Sinigaglia F, Guardiola J. MHC: orchestrating the immune response. *Immunol Today* 1995;16:8–11. [PubMed: 7880390]
35. Townsend A, Bodmer H. Antigen recognition by class I-restricted T lymphocytes. *Annu Rev Immunol* 1989;7:601–624. [PubMed: 2469442]
36. Hunt DF, Michel H, Dickinson TA, Shabanowitz J, Cox AL, Sakaguchi K, Appella E, Grey HM, Sette A. Peptides presented to the immune system by the murine class II major histocompatibility complex molecule I-A^d. *Science* 1992;256:1817–1820. [PubMed: 1319610]
37. Rock KL, Gramm C, Rothstein L, Clark K, Stein R, Dick L, Hwang D, Goldberg AL. Inhibitors of the proteasome block the degradation of most cell proteins and the generation of peptides presented on MHC class I molecules. *Cell* 1994;78:761–771. [PubMed: 8087844]
38. Yewdell JW, Bennink JR. Cell biology of antigen processing and presentation to major histocompatibility complex class I molecule-restricted T lymphocytes. *Adv Immunol* 1992;52:1–123. [PubMed: 1442305]
39. Falk K, Rötzschke O, Deres K, Metzger J, Jung G, Rammensee H-G. Identification of naturally processed viral nonapeptides allows their quantification in infected cells and suggests an allele-specific T cell epitope forecast. *J Exp Med* 1991;174:425–434. [PubMed: 1713253]
40. Rudensky AY, Preston-Hurlburt P, al-Ramadi BK, Rothbard J, Janeway CA Jr. Truncation variants of peptides isolated from MHC class II molecules suggest sequence motifs. *Nature* 1992;359:429–431. [PubMed: 1328884]
41. Bacik I, Cox JH, Anderson R, Yewdell JW, Bennink JR. TAP (transporter associated with antigen processing)-independent presentation of endogenously synthesized peptides is enhanced by endoplasmic reticulum insertion sequences located at the amino-but not carboxyl-terminus of the peptide. *J Immunol* 1994;152:381–387. [PubMed: 8283027]
42. Hartmann E, Sommer T, Prehn S, Gorlich D, Jentsch S, Rapoport TA. Evolutionary conservation of components of the protein translocation complex. *Nature* 1994;367:654–657. [PubMed: 8107851]

43. Restifo NP, Bacik I, Irvine KR, Yewdell JW, McCabe BJ, Anderson RW, Eisenlohr LC, Rosenberg SA, Bennink JR. Antigen processing *in vivo* and the elicitation of primary CTL responses. *J Immunol* 1995;154:4414–4422. [PubMed: 7722298]
44. Irvine KR, McCabe BJ, Rosenberg SA, Restifo NP. Synthetic oligonucleotide expressed by a recombinant vaccinia virus elicits therapeutic CTL. *J Immunol* 1995;154:4651–4657. [PubMed: 7722317]
45. Lin X, Dashti A, Schinazi RF, Tang J. Intracellular diversion of glycoprotein GP160 of human immunodeficiency virus to lysosomes as a strategy of AIDS gene therapy. *FASEB* 1993;7:1070–1080.
46. Rowell JF, Ruff AL, Guarnieri FG, Stavely-O'Carroll K, Lin X, Tang J, August T, Siliciano RF. Lysosome-associated membrane protein-1 mediated targeting of the HIV-1 envelope protein to an endosomal/lysosomal compartment enhances its presentation to MHC class II-restricted T cells. *J Immunol* 1995;155:1818–1828. [PubMed: 7636236]
47. Harding CV, Geuze HJ. Class II MHC molecules are present in macrophage lysosomes and phagolysosomes that function in the phagocytic processing of *Listeria monocytogenes* for presentation to T cells. *J Cell Biol* 1992;119:531–534. [PubMed: 1400590]
48. Colombo MP, Forni G. Cytokine gene transfer in tumor inhibition and tumor therapy: where are we now? *Immunol Today* 1994;15:48–51. [PubMed: 8155261]
49. Deres K, Schild H, Wiesmüller K-H, Jung G, Rammensee H-G. *In vivo* priming of virus-specific cytotoxic T lymphocytes with synthetic lipopeptide vaccine. *Nature* 1989;342:561–564. [PubMed: 2586628]
50. Schulz M, Zinkernagel RM, Hengartner H. Peptide-induced antiviral protection by cytotoxic T cells. *Proc Natl Acad Sci USA* 1991;88:991–993. [PubMed: 1992491]
51. Harty JT, Bevan MJ. CD8+ T cells specific for a single nonamer epitope of *Listeria monocytogenes* are protective *in vivo*. *J Exp Med* 1992;175:1531–1538. [PubMed: 1375265]
52. Nair S, Zhou F, Reddy R, Huang L, Rouse BT. Soluble proteins delivered to dendritic cells via pH-sensitive liposomes induce primary cytotoxic T lymphocyte responses *in vitro*. *J Exp Med* 1992;175:609–612. [PubMed: 1531064]
53. Romero P, Eberl G, Casanova JL, Cordey AS, Widmann C, Luescher IF, Corradin G, Maryanski JL. Immunization with synthetic peptides containing a defined malaria epitope induces a highly diverse cytotoxic T lymphocyte response. Evidence that two peptide residues are buried in the MHC molecule. *J Immunol* 1992;148:1871–1878. [PubMed: 1541825]
54. Zhou F, Rouse BT, Huang L. Prolonged survival of thymoma-bearing mice after vaccination with a soluble protein antigen entrapped in liposomes: a model study. *Cancer Res* 1992;52:6287–6291. [PubMed: 1384958]
55. Shirai M, Pendleton CD, Ahlers J, Takeshita T, Newman M, Berzofsky JA. Helper-cytotoxic T lymphocyte (CTL) determinant linkage required for priming of anti-HIV CD8+ CTL *in vivo* with peptide vaccine constructs. *J Immunol* 1994;152:549–555. [PubMed: 8283036]
56. Minev BR, McFarland BJ, Spiess PJ, Rosenberg SA, Restifo NP. Insertion signal sequence fused to minimal peptides elicits specific CD8+ T-cell responses and prolongs survival of thymoma-bearing mice. *Cancer Res* 1994;54:4155–4161. [PubMed: 7518351]
57. Takahashi H, Nakagawa Y, Yokomuro K, Berzofsky JA. Induction of CD8+ cytotoxic T lymphocytes by immunization with syngeneic irradiated HIV-1 envelope derived peptide-pulsed dendritic cells. *Int Immunol* 1993;5:849–857. [PubMed: 8398980]
58. Boehncke WH, Takeshita T, Pendleton CD, Houghten RA, Sadegh-Nasseri S, Racioppi L, Berzofsky JA, Germain RN. The importance of dominant negative effects of amino acid side chain substitution in peptide-MHC molecule interactions and T cell recognition. *J Immunol* 1993;150:331–341. [PubMed: 8093457]
59. Lipford GB, Bauer S, Wagner H, Heeg K. Peptide engineering allows cytotoxic T-cell vaccination against human papilloma virus tumor antigen, E6. *Immunology* 1995;84:298–303. [PubMed: 7751006]
60. Moss B. Vaccinia virus: a tool for research and vaccine development. *Science* 1991;252:1662–1667. [PubMed: 2047875]

61. Estin CD, Stevenson US, Plowman GD, Hu S-L, Sridham P, Hellstrom I, Brown JP, Hellstrom KE. Recombinant vaccinia virus against the human melanoma antigen p97 for use in immunotherapy. *Proc Natl Acad Sci USA* 1988;85:1052–1056. [PubMed: 3422478]
62. Hu S-L, Plowman GD, Sridhar P, Stevenson US, Brown JP, Estin C. Characterization of a recombinant vaccinia virus expressing human melanoma-associated antigen p97. *J Virol* 1988;62:176–180. [PubMed: 3334743]
63. Kantor J, Irvine K, Abrams S, Kaufman H, DiPietro J, Schlom J. Antitumor activity and immune responses induced by a recombinant carcinoembryonic antigen-vaccinia virus vaccine. *J Natl Cancer Inst* 1992;84:1084–1091. [PubMed: 1619682]
64. Kaufman H, Schlom J, Kantor J. A recombinant vaccinia virus expressing human carcinoembryonic antigen (CEA). *Int J Cancer* 1991;48:900–907. [PubMed: 1860736]
65. Clertant P, Kieny MP, Lecocq JP, Guizani I, Chambon P, Cuzin F, Lathe R. Recombinant polyoma-vaccinia viruses: T antigen expression vectors and anti-tumor immunization agents. *Biochimie* 1988;70:1075–1087. [PubMed: 2852508]
66. Hareuveni M, Gautier C, Kieny M-P, Wreschner D, Chambon P, Lathe R. Vaccination against tumor cells expressing breast cancer epithelial tumor antigen. *Proc Natl Acad Sci USA* 1990;87:9498–9502. [PubMed: 2251291]
67. Bernards R, Destree A, McKenzie S, Gordon E, Weinberg RA, Panicali D. Effective tumor immunotherapy directed against an oncogene-encoded product using a vaccinia virus vector. *Proc Natl Acad Sci USA* 1987;84:6854–6858. [PubMed: 3477812]
68. McCabe BJ, Irvine KR, Nishimura MI, Yang JC, Spiess PJ, Shulman EP, Rosenberg SA, Restifo NP. Minimal determinant expressed by a recombinant vaccinia virus elicits therapeutic antitumor cytolytic T lymphocyte responses. *Cancer Res* 1995;55:1741–1747. [PubMed: 7536130]
69. Mackett M, Smith GL, Moss B. Vaccinia virus: a selectable eukaryotic cloning and expression vector. *Proc Natl Acad Sci USA* 1982;79:7415–7419. [PubMed: 6296831]
70. Rodriguez JF, Smith GL. Inducible gene expression from vaccinia virus vectors. *Virology* 1990;177:239–250. [PubMed: 2191497]
71. Coupar BEH, Andrew ME, Both GW, Boyle DB. Temporal regulation of influenza hemagglutinin expression in vaccinia virus recombinants and effects on the immune response. *Eur J Immunol* 1986;16:1479–1487. [PubMed: 3493144]
72. Cooney EL, Collier AC, Greenberg PD, Coombs RW, Zarling J, Arditti DE, Hoffman MC, Hu SL, Corey L. Safety of and immunological response to a recombinant vaccinia virus vaccine expressing HIV envelope glycoprotein. *Lancet* 1991;337:567–572. [PubMed: 1671940]
73. Graham BS, Matthews TJ, Belshe RB, Clements ML, Dolin R, Wright PF, Gorse GJ, Schwartz DH, Keefer MC, Bolognesi DP. Augmentation of human immunodeficiency virus type 1 neutralizing antibody by priming with gp160 recombinant vaccinia and boosting with rgp160 in vaccinia-naïve adults. The NIAID AIDS Vaccine Clinical Trials Network. *J Infect Dis* 1993;167:533–537. [PubMed: 8095059]
74. Battegay M, Moskophidis D, Waldner H, Brundler MA, Fung-Leung WP, Mak TW, Hengartner H, Zinkernagel RM. Impairment and delay of neutralizing antiviral antibody responses by virus-specific cytotoxic T cells. *J Immunol* 1993;151:5408–5415. [PubMed: 7693811]
75. McElrath MJ, Corey L, Berger D, Hoffman MC, Klucking S, Dragavon J, Peterson E, Greenberg PD. Immune responses elicited by recombinant vaccinia-human immunodeficiency virus (HIV) envelope and HIV envelope protein: analysis of the durability of responses and effect of repeated boosting. *J Infect Dis* 1994;169:41–47. [PubMed: 8277196]
76. Baxby D, Paoletti E. Potential use of non-replicating vectors as recombinant vaccines. *Vaccine* 1992;10:8–9. [PubMed: 1311489]
77. Somogyi P, Frazier J, Skinner MA. Fowlpox virus host range restriction: gene expression, DNA replication, and morphogenesis in nonpermissive mammalian cells. *Virology* 1993;197:439–444. [PubMed: 8212580]
78. Taylor J, Weinberg R, Languet B, Desmettre P, Paoletti E. Recombinant fowlpox virus inducing protective immunity in non-avian species. *Vaccine* 1988;6:497–503. [PubMed: 2854338]

79. Wang M, Bronte V, Chen PW, Gritz L, Panicali D, Rosenberg SA, Restifo NP. Active immunotherapy of cancer with a nonreplicating recombinant fowlpox virus encoding a model tumor-associated antigen. *J Immunol* 1995;154:4685–4692. [PubMed: 7722321]
80. Taylor J, Meignier B, Tartalia J, Languet B, VanderHoeven J, Franchinni G, Trimarchi C, Paoletti E. Biological and immunogenic properties of a canarypox-rabies recombinant, ALVAC-RG (vCP65) in non-avian species. *Vaccine* 1995;13:539–549. [PubMed: 7483774]
81. Taylor J, Weinberg R, Tataglia J, Richardson C, Alkhatib G, Briedis D, Appel M, Norton E, Paoletti E. Non-replicating viral vectors as potential vaccines: recombinant canarypox virus expressing measles virus fusion (F) and hemagglutinin (HA) glycoproteins. *Virology* 1992;187:321–328. [PubMed: 1736535]
82. Cadoz M, Strady A, Meignier B, Taylor J, Tartaglia J, Paoletti E, Plotkin S. Immunisation with canarypox virus expressing rabies glycoprotein. *Lancet* 1992;339:1429–1432. [PubMed: 1351126]
83. Cox WI, Tartaglia J, Paoletti E. Induction of cytotoxic T lymphocytes by recombinant canarypox (ALVAC) and attenuated vaccinia (NYVAC) viruses expressing the HIV-1 envelope glycoprotein. *Virology* 1993;195:845–850. [PubMed: 8337851]
84. Meyer H, Sutter G, Mayr A. Mapping of deletions in the genome of the highly attenuated vaccinia virus MVA and their influence on virulence. *J Gen Virol* 1991;72:1031–1038. [PubMed: 2033387]
85. Pinto VB, Rock KL. Characterization of the proliferative response of a CD4-8-thymic T lymphoma cell line to stimulation by thymic cellular elements. *J Immunol* 1991;147:42–49. [PubMed: 1828825]
86. Graham FL, Prevec L. Adenovirus-based expression vectors and recombinant vaccines. *Biotechnology* 1992;20:363–390. [PubMed: 1318139]
87. Berkner KL. Expression of heterologous sequences in adenoviral vectors. *Curr Top Microbiol Immunol* 1992;158:39–66. [PubMed: 1582245]
88. Irwin MJ, Laube LS, Austin M, Chada S, Anderson C, Townsend K, Jolly DJ, Warner JF. Direct injection of a recombinant retroviral vector induces human immunodeficiency virus-specific immune responses in mice and nonhuman primates. *J Virol* 1994;68:5036–5044. [PubMed: 8035504]
89. Stover CK, de la Cruz VF, Fuerst TR, Burlein JE, Benson LA, Bennett LT, Bansal GP, Young JF, Lee MH, Hatfull GF, Snapper SB, Barletta RG, Jacobs WR Jr, Bloom BR. New use of BCG for recombinants vaccines. *Nature* 1991;351:456–460. [PubMed: 1904554]
90. Jacobs WRJ, Tuckman M, Bloom BR. Introduction of foreign DNA into mycobacteria using a shuttle plasmid. *Nature* 1987;327:532–535. [PubMed: 3473289]
91. Snapper SP, Lugosi L, Jekkel A, Melton RE, Kieser T, Bloom BR, Jacobs WR. Lysogeny and transformation in mycobacteria: Stable expression of foreign genes. *Proc Natl Acad Sci USA* 1988;85:6987–6991. [PubMed: 2842799]
92. Connell ND, Medina-Acosta E, McMaster WR, Bloom BR, Russell DG. Effective immunization against cutaneous leishmaniasis with recombinant bacille Calmette-Guerin expressing the *Leishmania* surface proteinase gp63. *Proc Natl Acad Sci USA* 1993;90:11473–11477. [PubMed: 8265576]
93. Stover CK, Bansal GP, Hanson MS, Burlein JE, Palaszynski SR, Young JF, Koenig S, Young DB, Sadziene A, Barbour AG. Protective immunity elicited by recombinant BCG expressing outer surface protein A (OspA) lipoprotein: A candidate Lyme Disease vaccine. *J Exp Med* 1993;178:197–209. [PubMed: 8315378]
94. Simon MM, Schaible UE, Karmer MD, Eckerson C, Museteanu C, Muller-Hermelink HK, Wallich R. Recombinant outer surface protein A from *Borrelia burgdorferi* induces antibodies protective against spirochetal infection in mice. *J Inf Dis* 1991;164:123–132. [PubMed: 1829104]
95. Tilney LG, Portnoy DA. Actin filaments and the growth, movement, and spread of the intracellular bacterial parasite, *Listeria monocytogenes*. *J Cell Biol* 1989;109:1597. [PubMed: 2507553]
96. Ikonomidis G, Paterson Y, Kos FJ, Portnoy DA. Delivery of a viral antigen to the class I processing and presentation pathway by *Listeria monocytogenes*. *J Exp Med* 1994;180:2209–2218. [PubMed: 7964496]
97. Schafer R, Portnoy DA, Brassell SA, Paterson Y. Induction of a cellular immune response to a foreign antigen by a recombinant *Listeria monocytogenes* vaccine. *J Immunol* 1992;149:53–59. [PubMed: 1607662]

98. Pan Z-K, Ikonomidis G, Lazenby A, Pardoll D, Paterson Y. A recombinant *Listeria monocytogenes* vaccine expressing a model tumour antigen protects mice against lethal tumour cell challenge and causes regression of established tumours. *Nature Med* 1995;1:471–480. [PubMed: 7585097]
99. Wolff JA, Malone RW, Williams P, Chong W, Acsadi G, Jani A, Felgner PL. Direct gene transfer into mouse muscle *in vivo*. *Science* 1990;247:1465–1468. [PubMed: 1690918]
100. Davis HL, Whalen RG, Demeneix BA. Direct gene transfer into skeletal muscle *in vivo*: factors affecting efficiency of transfer and stability of expression. *Hum Gene Ther* 1993;4:151–159. [PubMed: 8494924]
101. Williams RS, Johnston SA, Riedy M, DeVit MJ, McElligott SG, Sanford JC. Introduction of foreign genes into tissues of living mice by DNA-coated microprojectiles. *Proc Natl Acad Sci USA* 1991;88:2726–2730. [PubMed: 2011582]
102. Mielke ME, Ehlers S, Hahn H. T cell subsets in delayed-type hypersensitivity, protection, and granuloma formation in primary and secondary *Listeria* infection in mice: superior role of Lyt2+ cells in acquired immunity. *Infect Immun* 1988;56:1920. [PubMed: 2969373]
103. Eisenbraun MD, Fuller DH, Haynes JR. Examination of parameters affecting the elicitation of humoral immune responses by particle bombardment-mediated genetic immunization. *DNA Cell Biol* 1993;12:791–797. [PubMed: 8216850]
104. Pertmer TM, Eisenbraun MD, McCabe D, Prayaga SK, Fuller DH, Haynes JR. Gene gun-based nucleic acid immunization: elicitation of humoral and cytotoxic T lymphocyte responses following epidermal delivery of nanogram quantities of DNA. *Vaccine*. 1995in press
105. Yang N-S, Burkholder J, Roberts B, Martinelli B, McCabe D. *In vivo* and *in vitro* gene transfer to mammalian somatic cells by particle bombardment. *Proc Natl Acad Sci USA* 1990;87:9568–9572. [PubMed: 2175906]
106. Tang D, DeVit M, Johnston SA. Genetic immunization is a simple method for eliciting an immune response. *Nature* 1992;356:152–154. [PubMed: 1545867]
107. Fuller DH, Haynes JR. A qualitative progression in HIV type 1 glucoprotein 120-specific cytotoxic cellular and humoral immune responses in mice receiving a DNA-based glycoprotein 120 vaccine. *AIDS Res Hum Retrovir* 1994;10:1433. [PubMed: 7888198]
108. Ulmer JB, Donnelly JJ, Parker SE, Rhodes GH, Felgner PL, Dwarki VJ, Gromkowski SH, Deck RR, DeWitt CM, Friedman A. Heterologous protection against influenza by injection of DNA encoding a viral protein. *Science* 1993;259:1745–1749. [PubMed: 8456302]
109. Wang B, Ugen EU, Srikantan V, Agadjanyan MG, Dang K, Refaeli Y, Sato A, Boyer J, Williams WV, Weiner DB. Gene inoculation generates immune responses against human immunodeficiency virus type 1. *Proc Natl Acad Sci USA* 1993;90:4156–4160. [PubMed: 8483929]
110. Conry RM, LoBuglio AF, Loechel F, Moore SE, Sumerel LA, Barlow DL, Curiel DT. A carcinoembryonic antigen polynucleotide vaccine has *in vivo* antitumor activity. *Gene Ther* 1995;2:59–65. [PubMed: 7712333]
111. Wang B, Merva M, Dang K, Ugen KE, Williams WV, Weiner DB. Immunization by direct DNA inoculation induces rejection of tumor cell challenge. *Hum Gene Ther* 1995;6:407–418. [PubMed: 7612698]
112. Bluestone J. New perspectives of CD28-B7-mediated T cell costimulation. *Immunity* 1995;2:555–559. [PubMed: 7540940]
113. Chen L, Ashe S, Brady WA, Hellstrom I, Hellstrom KE, Ledbetter JA, McGowan P, Linsley PS. Costimulation of antitumor immunity by the B7 counterreceptor for the T lymphocyte molecules CD28 and CTLA-4. *Cell* 1992;71:1093–1102. [PubMed: 1335364]
114. Chen L, McGowan P, Ashe S, Johnson J, Li Y, Hellstrom I, Hellstrom KE. Tumor immunogenicity determines the effect of B7 costimulation on T cell-mediated tumor immunity. *J Exp Med* 1994;179:523–532. [PubMed: 7507508]
115. Li Y, McGowan P, Hellstrom I, Hellstrom KE, Chen L. Costimulation of tumor-reactive CD4+ and CD8+ T lymphocytes by B7, a natural ligand for CD28, can be used to treat established mouse melanoma. *J Immunol* 1994;153:421–428. [PubMed: 7515929]
116. Yang G, Hellstrom KE, Hellstrom I, Chen L. Antitumor immunity elicited by tumor cells transfected with B7-2, a second ligand for CD28/CTLA-4 costimulatory molecules. *J Immunol* 1995;154:2794–2800. [PubMed: 7533183]

117. Hodge JW, Abrams S, Schlom J, Kantor JA. Induction of antitumor immunity by recombinant vaccinia viruses expressing B7-1 or B7-2 costimulatory molecules. *Cancer Res* 1994;54:5552–5555. [PubMed: 7522961]
118. Hodge JW, McLaughlin JP, Abrams SI, Shupert L, Schlom J, Kantor JA. Admixture of a recombinant vaccinia virus containing the gene for the costimulatory molecule B7 and a recombinant vaccinia virus containing a tumor-associated antigen gene results in enhanced specific T-cell responses and antitumor immunity. *Cancer Res* 1995;55:3598–3603. [PubMed: 7543017]
119. Bronte V, Tsung K, Rao JB, Chen PW, Wang M, Rosenberg SA, Restifo NP. IL-2 enhances the function of recombinant poxvirus-based vaccines in the treatment of established pulmonary metastases. *J Immunol* 1995;154:5282–5292. [PubMed: 7730632]
120. Mosmann TR. Properties and functions of IL-10. *Adv Immunol* 1994;56:1–26. [PubMed: 8073945]
121. Kennedy MK, Picha KS, Shanebeck KD, Anderson DM, Grabstein KH. Interleukin-12 regulates the proliferation of Th1, but not Th2 or Th0, clones. *Eur J Immunol* 1994;24:2271–2278. [PubMed: 7925555]

Figure 1.

Transcriptional and translational regulation of foreign gene products within different recombinant vectors. Some recombinant vectors, such as bacterial vectors, provide their own transcriptional and translational machinery for the production of protein products. Others, such as recombinant pox viruses, transcribe their genes using viral polymerases but rely on the host for translation of the mRNA produced. Recombinant adenoviruses or DNA-based immunization only supply DNA and depend on host polymerases to transcribe and translate the heterologous protein.

Table 1

Candidate vaccines in the treatment of cancer

Vaccine	Example	Advantage(s)
Whole tumor cells	Physically altered (γ -irradiated, viral oncolysates)	Expression of multiple antigens
	Gene modified (IL-2, TNF- α , IFN- γ) (GM-CSF, B7-1)	Expression of multiple antigens in the context of immunomodulatory molecules
Peptides/proteins	Peptides in incomplete Freund's adjuvant	Pure, single epitope
Plasmid DNA	Intramuscular injection, 'Gene gun' injection	Easily purified No anamnestic responses to vector
Recombinant organisms	Bacille Calmette-Guerin, <i>Listeria monocytogenes</i>	Produce high quantities of heterologous proteins Unique biological properties Powerful adjuvant activity
Recombinant viruses	Vaccinia, fowlpox adenovirus, retrovirus	Produce high quantities of heterologous proteins, Exploits intracellular trafficking pathways. Directs synthesis of integral membrane glycoproteins (including costimulatory molecules) Powerful adjuvant activity