Review

Gene therapy for carcinoma of the breast
Genetic immunotherapy

Theresa V Strong

University of Alabama at Birmingham, Birmingham, Alabama, USA

Received: 10 November 1999
Accepted: 18 November 1999
Published: 17 December 1999

Breast Cancer Res 2000, **2**:15–21

Abstract

Advances in gene transfer technology have greatly expanded the opportunities for developing immunotherapy strategies for breast carcinoma. Genetic immunotherapy approaches include the transfer of genes encoding cytokines and costimulatory molecules to modulate immune function, as well as genetic immunization strategies which rely on the delivery of cloned tumor antigens. Improved gene transfer vectors, coupled with a better understanding of the processes that are necessary to elicit an immune response and an expanding number of target breast tumor antigens, have led to renewed enthusiasm that effective immunotherapy may be achieved. It is likely that immunotherapeutic interventions will find their greatest clinical application as adjuvants to traditional first-line therapies, targeting micrometastatic disease and thereby reducing the risk of cancer recurrence.

Keywords: cytokine, gene transfer, tumor antigen, vaccine

Introduction

A central issue in the management of women with breast cancer is the prevention of metastatic disease. Although primary surgical treatment is generally effective at controlling local disease, many patients have micrometastases at the time of diagnosis. A substantial proportion of women diagnosed with breast cancer will have a subsequent recurrence of disease, and it is in these women in whom significant morbidity and mortality occurs. Adjuvant therapies with hormones or chemotherapy have resulted in a modest decrease in the relapse rate, but novel approaches are needed. A primary difficulty lies in defining a treatment that will effectively destroy disseminated tumor cells without significant toxicity to the patient. Immunotherapy attempts to achieve this goal by recruiting the host's immune system to identify and destroy aberrant tumor cells. Studies of the interaction of tumor cells with cells of the immune system has led to the development of novel, more rational strategies for immunotherapy.

Cancer immunity and evasion of immune response

It has long been apparent that tumor cells exhibit some degree of immunogenicity, and attempts to enhance the immune response to tumor cells date back more than 90 years [1,2]. Cancer immunotherapy strategies are based on eliciting or augmenting a specific host immune response to tumor-associated antigens (TAAs) that are

APC = antigen presenting cell; CEA = carcinoembryonic antigen; CTL = cytotoxic T lymphocyte; DC = dendritic cell; GM-CSF = granulocyte–macrophage colony-stimulating factor; HLA = human leukocyte antigen; IL = interleukin; KLH = keyhole limpet haemocyanin; MHC = major histocompatibility complex; TAA = tumor-associated antigen; TAP = transporter associated with antigen processing.

present on tumor cells. Such approaches are particularly aimed at enhancing the function of the cellular immune response, as it is believed that killing of tumor cells is primarily mediated through the action of T cells.

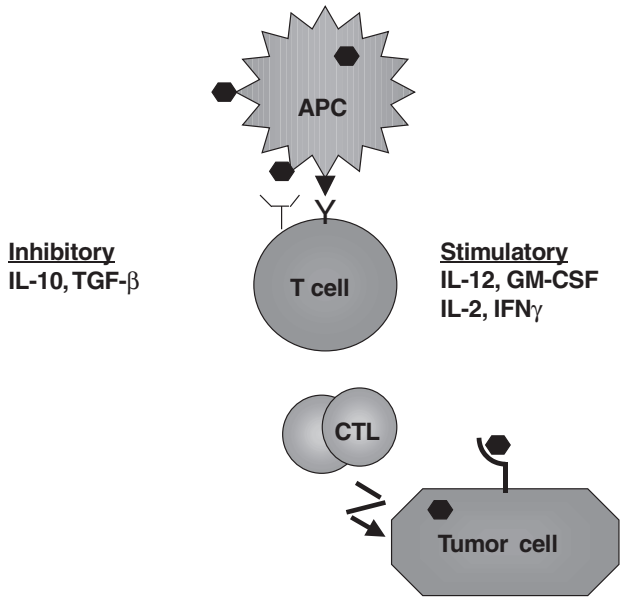
There is considerable evidence that the immune system can recognize tumor cells by virtue of TAAs, and a limited immune response to many of these antigens is detectable in patients. Breast cancer patients exhibit both circulating antibodies and cytotoxic T lymphocytes (CTLs) that are specific for breast tumor antigens, including HER-2/neu (erbB-2) [3,4], MAGE-1 [5], and MUC-1 [6]. In addition, both $CD4^+$ and $CD8^+$ T cells have been identified as components of breast tumor infiltrating lymphocytes [7]. Despite these specific immune responses, tumor cells manage to evade detection and/or destruction. Recent advances in tumor immunology [8•] have provided a more complete understanding of the interaction of tumors with the immune system, and have delineated the diverse mechanisms by which tumor cells circumvent the immune response.

Tumor cells themselves may exhibit altered properties as a means to avoid T-cell recognition. These can include downregulation of expression of specific antigens [9,10], or major histocompatibility complex (MHC) molecules [11,12]. Defects in the antigen-processing machinery, specifically the peptide transporter associated with antigen processing (TAP) molecules, may lead to an overall decrease in immunogenic peptides on the tumor cell surface [13]. In breast cancer, downregulation of TAP expression with concurrent loss of human leukocyte antigen (HLA) class I expression is common in high-grade lesions [14•]. As an alternative mechanism, tumors may influence T-cell responsiveness by secreting immunosuppressive molecules such as interleukin (IL)-10 and transforming growth factor-β [15,16], or by directly killing Fas-positive infiltrating T cells through expression of the Fas ligand [17,18]. The interaction of Fas with Fas ligand induces apoptosis in the infiltrating lymphocytes. Finally, a common reason that T cells do not respond to tumors may be that TAAs are presented by antigen-presenting cells in such a way that anergy and tolerance is induced, rather than T-cell activation [19,20].

The importance of appropriate immunostimulatory molecules during antigen presentation has recently been appreciated. The long-standing immune surveillance theory holds that immune responsiveness is based on the recognition of self versus nonself [21], and that tolerance to self is established in the neonatal period. That theory has been challenged because it has become more apparent that the environment in which an antigen is presented is critical in determining the type and extent of immune response induced [22]. The danger theory [23] suggests that it is the release of alarm signals, induced by cells experiencing stress, that promotes a potent immune response. Therefore, the presence of costimulatory molecules that activate T cells during antigen presentation is critical to the development of a robust immune response. Effective antigen-presenting cells express the costimulatory molecule B7 in conjunction with the antigen on the cell surface. This molecule also engages the T cell, providing the necessary signal to promote activation. The effectiveness of this response is modulated by the presence of cytokines, with stimulatory cytokines such as IL-12 enhancing T-cell response (Fig. 1). Without appropriate stimulatory signals, T cells encountering an antigen move to anergy or undergo apoptosis.

This new understanding of the potential defects in the elicitation of tumor-specific immune response has led to the development of strategies that address these deficiencies. Furthermore, these observations suggest that it should be possible, with proper manipulation of the immune cells and the local cytokine milieu, to induce an immune response to both self and nonself molecules. As the processes required for autologous tumor rejection become

Figure 1



The microenvironment is critical to the stimulation of an effective T-cell-mediated antitumor immune response. Presentation of the antigen (⬣) to T cells by antigen-presenting cells (APCs) must include the engagement of costimulatory molecules such as B7 (▼) on the APC with CD28 (**Y**) on the T cell. Detection of antigen in the absence of this signal can result in tolerance. Release of cytokines including interleukin (IL)-12, granulocyte–macrophage colony-stimulating factor (GM-CSF) (by APCs), IL-2 and interferon (IFN)γ (by T cells) stimulate a strong cellular immune response, whereas local production of IL-10 or transforming growth factor (TGF)-β blunts T cell responsiveness. Finally, for cytotoxic T lymphocytes (CTLs) to be able to recognize their targets, the tumor cells have functional major histocompatibility complex class I tumor antigen presentation (ϟ).

better characterized, new strategies are being developed to potentiate the immunogenicity of tumor cells.

Whole cell tumor vaccines

A variety of strategies that rely on transfer of cloned genes have been developed to elicit or enhance host immune response to tumor cells. Transfer of genes that encode immunostimulatory molecules (cytokines and/or costimulatory molecules) directly into tumor cells is one means to enhance their immunogenicity. Several cytokines have been evaluated by this approach. Initial studies focused on IL-2. Although it was shown to be effective in boosting antitumor immunity, IL-2 can be associated with unacceptable toxicity when delivered systemically [24]. To overcome the toxicity of systemic delivery, direct *ex vivo* transduction of tumor cells with cytokine-encoding complementary DNAs has been explored in mouse models of mammary carcinoma [25,26]. After irradiation, transduced cells may be used as a vaccine, providing a scenario in which tumor antigens are available in an environment of locally high concentrations of the immunostimulatory molecules. The presence of this cytokine allows direct activation of $CD8^+$ cytolytic T cells (CTLs), bypassing the need for $CD4^+$ help [27]. In addition to IL-2, a variety of other cytokines have been explored in this manner. Interferon-γ has pleiotropic effects, including upregulation of MHC molecules and the recruitment of cells of the immune system [28,29]. Granulocyte–macrophage colony-stimulating factor (GM-CSF) [30] and IL-12 [31] have also shown efficacy in animal model studies. GM-CSF is notable because it promotes dendritic cell (DC) differentiation, and may thus mediate the most effective presentation of tumor antigens (see below). Some encouraging initial results have been reported using this cytokine in prostate cancer patients [32]. IL-12 is also of interest because it particularly enhances the cellular arm of the immune system.

To further increase the effectiveness of whole cell tumor vaccines, several groups have combined immunostimulatory genes for transduction of tumor cells. Transduction of tumor cells with different cytokines [33] or cytokines with the B7 costimulatory gene [34,35] has proved to be more effective than using a single cytokine alone. Genes that encode cytokines delivered in combination with a chemokine [36••] also enhance immune responses, presumably as a result of more efficient recruitment of relevant lymphocytes to the site. Augmentation of cytokine effectiveness may also be achieved by interfering with inhibitory signals delivered to T cells. This was accomplished by blocking the interaction of CTL-antigen-4 on T cells with B7 on the antigen-presenting cell. Blocking this inhibitory signal while delivering GM-CSF transduced mammary carcinoma cells led to a more robust antitumor response [37••]. The manipulation of the antigen-presenting environment has thus proved to be an effective means of generating a potent antitumor immune response in otherwise weakly immunogenic models. The collective success of these animal model studies has provided the basis for the development of gene therapy clinical trials using tumor cells transduced with immunostimulatory molecules [38]. These trials will begin to investigate whether these strategies can be successfully translated into the clinical setting.

Target antigens for breast cancer immunotherapy

Although the use of cell-based vaccines as described above is advantageous in that it does not depend on defining the relevant tumor rejection antigens, the use of cloned tumor antigens in a vaccine strategy offers some advantages compared with whole cell preparations. Dose of antigen, an important parameter in the induction of an immune response, is more readily controlled with delivery of cloned tumor antigens. Furthermore, there are fewer safety concerns associated with a defined tumor vaccine, because the likelihood of eliciting an immune response to irrelevant proteins is decreased. Genetic modification of the encoded tumor antigen to optimize presentation is also readily accomplished when using cloned tumor antigens [39]. Finally, the use of a defined tumor antigen allows for direct monitoring of both T-cell and humoral immune responses to the antigen of interest. This end point allows valuable information to be gathered from early clinical trials, even if the therapy itself does not result in noticeable effects on tumor burden.

Potential targets currently under investigation for vaccination in breast carcinoma include the HER-2/neu protein [3,4,40,41], carcinoembryonic antigen (CEA) [41], MAGE-1 [5], and MUC-1 [6,42,43]. These antigens have been pursued on the basis of the high levels of expression in breast tumor tissue compared with normal tissue, as well as an understanding of the epitopes recognized by CTLs. Mutant cellular proteins such as mutant p53 may also provide useful targets for immunotherapy. In addition to these relatively well characterized antigen targets, a number of potential new targets are being studied that may have relevance to breast cancer immunotherapy.

One new approach to tumor antigen identification focuses on identifying genes that encode MHC class II-restricted antigens [44]. Initially used to identify a melanoma antigen, this general approach may be applied for the definition of breast TAAs that are recognized by $CD4^+$ cells, expanding the targets for immunotherapy. Another approach seeks to identify TAAs based on a serologic assay rather than T-cell responsiveness. This approach, termed 'serologic identification of antigens by recombinant expression cloning' (SEREX), is based on the observation that cancer patients exhibit circulating antibodies directed towards tumor antigens, and that these antibodies may provide a useful

reagent for the identification new tumor antigens [45,46]. Serologic identification of antigens by recombinant expression cloning analysis has greatly expanded the list of potential targets for breast cancer immunotherapy [47•], but whether these antigens will be able to elicit a potent T-cell response remains to be determined. In this regard, simultaneous stimulation of both cellular and humoral immune responses has been demonstrated and is not unexpected, because both rely on helper T-cell function [48•]. The expanding list of antigen targets will be of considerable importance in the development of effective immunotherapies, because each of these antigens is expressed only on a portion of breast tumors. Simultaneous immunization against multiple antigens is likely to be most effective, because it will protect against clonal outgrowth of tumor cells downregulating a single target antigen.

Vectors for gene transfer

A variety of vectors have been employed for transfer of genes that encode tumor antigens or immunostimulatory molecules. These vectors may deliver genes to tumor cells expanded *ex vivo* for whole cell vaccination, to tumor cells *in vivo* via direct intratumoral injection, or to muscle or skin for immunization against cloned tumor antigens.

Retroviral-mediated gene transfer was the method of choice in most initial studies [49,50]. Although effective in animal models, these viruses are difficult to produce in high titers and are associated with a relatively low level of transduction.

Recombinant vaccinia virus is a potent stimulator of cellular and humoral immune response [51,52], suggesting potential of this vector in cancer immunotherapy applications. Immunization of mice with a recombinant vaccinia virus that encoded CEA resulted in a strong cellular and humoral immune response, and the animals exhibited a delayed type hypersensitivity in response to challenge with CEA-expressing tumor cells [53]. Another pox virus vector, the recombinant canarypox virus termed ALVAC, is unable to produce a productive infection in mammalian cells, but can direct high levels of transgene expression, making it a potentially useful vector for immunotherapy [54]. Based on preclinical studies, both recombinant vaccinia and ALVAC vectors are currently under investigation in phase 1 clinical trials for cancer immunotherapy [55,56].

Adenovirus is another viral vector commonly used for gene therapy applications that may be exploited for immunotherapy. This vector is readily prepared in high titers and mediates high levels of transient transgene expression in a variety of cell types. Unfortunately, pre-existing immunity to naturally occurring adenovirus is common, and may compromise the effectiveness of this vector when used for *in vivo* delivery [57].

To circumvent immune responses to viral vectors and to simplify vaccine production, nucleic acids that encode relevant tumor antigens or immunostimulatory molecules can be delivered by nonviral means. Liposomes may be used for this purpose [58], but genes may also be delivered 'naked'. The feasibility of such an approach was first recognized when DNA injected intramuscularly was found to be taken up by a small proportion of host cells, in which it may be expressed locally for extended periods of time [59]. In animal models, DNA immunization has been shown to elicit effective antitumor immune responses to CEA and neu [60–62]. Interestingly, the properties of plasmid DNA produced in a bacterial system, namely the lack of CpG dinucleotide methylation, acts as an immunostimulant in mammals, enhancing the immune response to the encoded antigen [63]. In addition to plasmid DNA, self-replicating RNA offers a potentially powerful method of antigen delivery [64•,65]. Again, the nucleic acid itself is immunostimulatory. In this case, the double-stranded RNA intermediate generated during the replication process may act as a 'danger' signal, amplifying the immune response. Apoptosis induced by this vector and subsequent uptake of apoptotic cells by DCs probably also contribute to the potent response.

In addition to direct intramuscular injection, biolistic delivery of naked DNA or RNA (using the 'gene gun') to the skin offers an alternative method of nonviral gene transfer for immunization [66]. The advantages of this method include the small amounts of nucleic acid needed for delivery, as well as ease of delivery. The disadvantage of this approach has been that this route of delivery favors the development of a T-helper 2, or predominantly antibody response, whereas a T-helper 1, or cellular immune response is preferred for cancer immunotherapy. This unfavorable characteristic may be surmountable by codelivery of cytokines that shift the immune response to a T-helper 1-type response [67•].

In summary, a number of vectors with diverse characteristics are available for gene delivery in the context of genetic immunotherapy. The choice of appropriate vector is dependent on the particular application; however, the vector development field is rapidly evolving. It is likely that refinements in vectors that improve targeting and transgene expression will further the field of genetic immunotherapy.

Dendritic cell-based vaccines

DCs have been recognized as important mediators of immune response. They are specialized antigen-presenting cells that are highly potent in their presentation of antigen to naïve or quiescent $CD4^+$ and $CD8^+$ T cells [68]. They capture, process, and present antigens in combination with MHC class I and II molecules, activating specific CTLs. This ability to stimulate CTLs directly and effectively makes DCs ideal targets to exploit for manipulation of the immune system for cancer immunotherapy purposes.

Cell culture techniques have evolved that now permit the *in vitro* generation of large numbers of DCs from bone marrow or peripheral blood mononuclear cells, making DC vaccination technically feasible. Recently published pilot clinical trials of antigenic protein or peptide-pulsed DCs in non-Hodgkin's lymphoma and melanoma have demonstrated the general safety of this approach, as well as some evidence of antigen-specific immune responses and occasional clinical tumor regressions [69,70]. A controlled study [71] in which healthy individuals were injected subcutaneously with autologous monocyte-derived DCs pulsed with keyhole limpet haemocyanin (KLH), tetanus toxoid, or an HLA-A2-restricted influenza matrix peptide demonstrated priming of $CD4^+$ T cells to KLH, boosting of tetanus toxoid-specific T-cell immunity, and increases in influenza peptide-specific $CD8^+$ T, respectively, whereas injection of antigens alone failed to immunize control individuals. Although these findings are preliminary, they suggest the potential for DC-mediated vaccination strategies.

In addition to peptide or protein pulsing into DCs, several studies have focused on delivering genes that encode tumor antigens to the DCs. One advantage offered by this approach is enhanced efficiency of MHC peptide loading compared with pulsing of DCs with intact protein. Furthermore, compared with peptide pulsing, transfer of the antigen-encoding gene and intracellular synthesis of the complete protein allows the host to select antigenic epitopes from the entire protein, rather than being restricted to a single epitope. Several promising gene transfer approaches exist that have been used to transduce DCs *ex vivo* and promote therapeutic tumor immunity in model systems. These include retroviral, poxvirus, and adenoviral vectors, a targeted adenoviral vector, as well as naked RNA [72–76,77•,78].

Conclusion

The prospects for successful immunotherapy of cancer have improved based on insights from a wide range of fields related to tumor immunology. Appreciation of the basis of poor immunogenicity of tumor cells, cloning and functional analysis of cytokines, and recognition of the critical cells and costimulatory molecules that are involved in immune recognition have facilitated the development of rational strategies to stimulate antitumor immune responses. In the coming years, the development of advanced generation gene transfer vectors should enhance our ability to target appropriate cells specifically and to achieve optimal transgene expression, and definition of breast tumor antigens will expand the targets for immunotherapy. These new developments will be brought to bear on the design of clinical trials of novel immunotherapies. Evaluation of these new genetic immunotherapy strategies in clinical trials that employ careful immune response analysis studies should guide further development in the field, allowing it to reach its full potential and offering new hope to those with breast cancer.

References

Articles of particular interest have been highlighted as:
- • of special interest
- •• of outstanding interest

1. Ottgen HF, Old LJ: **The history of cancer immunotherapy.** In: *Biological Therapy of Cancer, Principles and Practice*. Edited by DeVita VT, Helman S, Rosenberg SA. JB Lippincott, Philadelphia PA, 1991:87–90.

2. Nauts HC: **Bacteria and cancer: antagonisms and benefits.** *Cancer Surv* 1989, **8**:713–723.

3. Disis ML, Calenoff E, McLaughllun G, *et al*: **Existent T-cell and antibody immunity to HER-2/neu protein in patients with breast cancer.** *Cancer Res* 1994, **54**:16–20.

4. Disis ML, Pupa SM, Gralow JR, *et al*: **High-titer HER-2/neu protein-specific antibody can be detected in patients with early-stage breast cancer.** *J Clin Oncol* 1997, **15**:3363–3367.

5. Toso JF, Oei C, Oshidari F, *et al*: **MAGE-1-specific precursor cytotoxic T-lymphocytes present among tumor-infiltrating lymphocytes from a patient with breast cancer: characterization and antigen-specific activation.** *Cancer Res* 1996, **56**:16–20.

6. Ioannides CG, Fisk B, Jerome KR, *et al*: **Cytotoxic T cells from ovarian malignant tumors can recognize polymorphic epithelial mucin core peptides.** *J Immunol* 1993, **15**:3693–3703.

7. Whitford P, Mallon EA, George WD, Campbell AM: **Flow cytometric analysis of tumor infiltrating lymphocytes in breast cancer.** *Br J Cancer* 1990, **62**:971–975.

8. • Pardoll DM: **Cancer vaccines.** *Nature Med* 1998, **4 (suppl)**: 525–531.
An excellent review of the underlying principles and challenges in the development of effective cancer vaccines.

9. Uyttenhove C, Maryanski J, Boon T: **Escape of mouse mastocytoma P815 after nearly complete rejection is due to antigen-loss variants rather than immunosuppression.** *J Exp Med* 1983, **157**:1040–1052.

10. Wortzel RD, Philipps C, Schreiber H: **Multiple tumour-specific antigens expressed on a single tumour cell.** *Nature* 1983, **304**:165–167.

11. Hui K, Grosveld F, Festenstein H: **Rejection of transplantable AKR leukemia cells following MHC DNA-mediated cell transformation.** *Nature* 1984, **311**:750–752.

12. Wallich R, Bulbuc N, Hammerling GJ, *et al*: **Abrogation of metastatic properties of tumour cells by *de novo* expression of H-2K antigens following H-2 gene transfection.** *Nature* 1985, **315**:301–305.

13. Restifo NP, Esquivel F, Kawakami Y, *et al*: **Identification of human cancers deficient in antigen processing.** *J Exp Med* 1993, **177**:265–272.

14. • Vitale M, Rezzani R, Rodella L, *et al*: **HLA class I antigen and transporter associated with antigen processing (TAP1 and TAP2) down-regulation in high-grade primary breast carcinoma lesions.** *Cancer Res* 1998, **58**:737–742.
An important mechanism of escape from immune recognition by breast tumor cells is described.

15. Morisaki T, Katano M, Ikubo A, *et al*: **Immunosuppressive cytokine (IL-10, TGF-beta) gene expression in human gastric carcinoma tissues.** *J Surg Oncol* 1996, **63**:234–239.

16. McEarchern JA, Besselsen DG, Akporiaye ET: **Interferon gamma and antisense transforming growth factor beta transgenes synergize to enhance the immunogenicity of a murine mammary carcinoma.** *Cancer Immunol Immunother* 1999, **48**:63–70.

17. Hahne M, Rimoldi D, Schroter M, *et al*: **Melanoma cell expression of Fas(Apo-1/CD95) ligand: implications for tumor immune escape.** *Science*. 1996, **274**:1363–1366.

18. Gutierrez LS, Eliza M, Niven-Fairchild T, Naftolin F, Mor G: **The Fas/Fas-ligand system: a mechanism for immune evasion in human breast carcinomas.** *Br Cancer Res Treat* 1999, **54**:245–253.

19. Burkly LC, Lo D, Kanagawa O, Brinster RL, Flavell RA: **T-cell tolerance by clonal anergy in transgenic mice with nonlymphoid expression of MHC class II I-E.** *Nature* 1989, **342**:564–566.

20. Ohashi PS, Oehen S, Buerki K, *et al*: **Ablation of 'tolerance' and induction of diabetes by virus infection in viral antigen transgenic mice.** *Cell* 1991, **65**:305–317.

21. Burnet FM: **The concept of immunological surveillance.** *Prog Exp Tumor Res* 1970, **13**:1–27.

22. Ridge JP, Fuch EJ, Matzinger P: **Neonatal tolerance revisited: turning on newborn T cells with dendritic cells.** *Science* 1996, **271**: 1723–1726.

23. Matzinger P: **An innate sense of danger.** *Semin Immunol* 1998, **10**: 399–415.

24. Rosenberg SA, Lotze MT, Yang JC, *et al*: **Experience with the use of high-dose interleukin-2 in the treatment of 652 patients.** *Ann Surg* 1989, **210**:474–484.

25. Coveney E, Clary B, Iacobucci M, Philip R, Lyerly K: **Active immunotherapy with transiently transfected cytokine-secreting tumor cells inhibits breast cancer metastases in tumor-bearing animals.** *Surg* 1996, **120**:265–272.

26. Matory YL, Dorfman DM, Chen M, *et al*: **T cells mediate treatment of six-day-old cytokine-gene-transfected mouse mammary tumor.** *Pathobiology* 1999, **67**:3–11.

27. Fearon ER, Pardoll D, Itaya T, *et al*: **Interleukin-2 production by tumor cells bypasses T helper function in the generation of antitumor immune response.** *Cell* 1990, **60**:397–403.

28. Gansbacher B, Rosenthal FM, Zier K: **Retroviral vector-mediated cytokine-gene transfer into tumor cells.** *Cancer Invest* 1993, **11**:345–354.

29. Matory YL, Chen M, Dorfman DM, Williams A, Goedegebuure PS, Eberlein TJ: **Antitumor activity of three mouse mammary cancer cell lines after interferon-gamma gene transfection**. *Surgery* 1995, **118**:251–256.

30. Shi FS, Weber S, Gan J, Rakhmilevich AL, Mahvi DM: **Granulocyte–macrophage colony-stimulating factor (GM-CSF) secreted by cDNA-transfected tumor cells induces a more potent antitumor response than exogenous GM-CSF.** *Cancer Gene Ther* 1999, **6**:81–88.

31. Bramson JL, Hitt M, Addison CL, *et al*: **Direct intratumoral injection of an adenovirus expressing interleukin-12 induces regression and long-lasting immunity that is associated with highly localized expression of interleukin-12.** *Hum Gene Ther* 1996, **7**:1995–2002.

32. Simons JW, Mikhak B, Chang J-F, *et al*: **Induction of immunity to prostate cancer antigens: results of a clinical trials of vaccination with irradiated autologous prostate tumor cells engineered to secrete granulocyte-macrophage colony-stimulating factor using *ex vivo* gene transfer.** *Cancer Res* 1999, **59**:5160–5168.

33. Addison CL, Bramson JL, Hitt MM, *et al*: **Intratumoral coinjection of adenoviral vectors expressing IL-2 and IL-12 results in enhanced frequency of regression of injected and untreated distal tumors.** *Gene Ther* 1998, **5**:1400–1409.

34. Emtage PC, Wan Y, Bramson JL, Graham FL, Gauldie J: **A double recombinant adenovirus expressing the costimulatory molecule B7-1 (murine) and human IL-2 induces complete tumor regression in a murine breast adenocarcinoma model.** *J Immunol* 1998, **160**:2531–2538.

35. Hurwitz AA, Townsend SE, Yu TF, Wallin JA, Allison JP: **Enhancement of the anti-tumor immune response using a combination of interferon-gamma and B7 expression in an experimental mammary carcinoma.** *Int J Cancer* 1998, **77**:107–113.

36. •• Emtage PC, Wan Y, Hitt M, *et al*: **Adenoviral vectors expressing lymphotactin and interleukin 2 or lymphotactin and interleukin 12 synergize to facilitate tumor regression in murine breast cancer models.** *Hum Gene Ther* 1999, **10**:697–709.

The use of a chemokine represents a novel approach to enhance cytokine activation.

37. •• Hurwitz AA, Yu TF, Leach DR, Allison JP: **CTLA-4 blockade synergizes with tumor-derived granulocyte-macrophage colony-stimulating factor for treatment of an experimental mammary carcinoma.** *Proc Natl Acad Sci USA* 1998, **95**:10067–10071.

This paper demonstrates that manipulation of T-cell interaction with the antigen-presenting cell to favor activation enhances the immune response.

38. Anonymous: **Human gene marker/therapy clinical protocols.** *Hum Gene Ther* 1999, **10**:2037–2088.

39. Lin K-Y, Guarnieri FG, Staveley-O'Carroll KF, *et al*: **Treatment of established tumors with a novel vaccine that enhance major histocompatibility class II presentation of tumor antigen.** *Cancer Res* 1996, **56**:21–26.

40. Disis ML, Grabstein KH, Sleath PR, Cheever MA: **Generation of immunity to the HER-2/neu oncogenic protein in patients with breast and ovarian cancer using a peptide-based vaccine.** *Clin Cancer Res* 1999, **5**:1289–1297.

41. Kawashima I, Tsai V, Southwood S, *et al*: **Identification of HLA-A3-restricted cytotoxic T lymphocyte epitopes from carcinoembryonic antigen and HER-2/neu by primary *in vitro* immunization with peptide-pulsed dendritic cells.** *Cancer Res* 1999, **59**:431–435.

42. Henderson RA, Konitsky WM, Barratt-Boyes SM, Soares M, Robbins PD, Finn OJ. **Retroviral expression of MUC-1 human tumor antigen with intact repeat structure and capacity to elicit immunity *in vivo*.** *J Immunother* 1998, **21**:247–256.

43. Brossart P, Heinrich KS, Stuhler G, *et al*: **Identification of HLA-A2-restricted T-cell epitopes derived from the MUC1 tumor antigen for broadly applicable vaccine therapies.** *Blood* 1999, **93**:4309–4317.

44. Wang R-F, Wang X, Atwood AC, Topalian SL, Rosenberg SA: **Cloning genes encoding MHC class II restricted antigens: mutated CDC27 as a tumor antigen.** *Science* 1999, **284**:1351–1354.

45. Sahin U, Tureci O, Schmitt H, *et al*: **Human neoplasms elicit multiple specific immune responses in the autologous host.** *Proc Natl Acad Sci USA* 1995, **92**:11810–11813.

46. Old LJ, Chen YT: **New paths in human cancer serology.** *J Exp Med* 1998, **187**:1163–11677.

47. • Sahin U, Tureci O, Chen YT, *et al*: **Expression of multiple cancer/testis (CT) antigens in breast cancer and melanoma: basis for polyvalent CT vaccine strategies.** *Int J Cancer* 1998, **78**: 387–389.

A list of a new class of tumor-associated antigens is provided, that are identified on the basis of serologic immune response, with potential for breast cancer immunotherapy.

48. • Jager E, Chen YT, Drijfhout JW, *et al*: **Simultaneous humoral and cellular immune response against cancer-testis antigen NY-ESO-1: definition of human histocompatibility leukocyte antigen (HLA)-A2-binding peptide epitopes.** *J Exp Med* 1998, **187**: 265–270.

This paper demonstrates that B-cell and T-cell responses are not exclusive. A cancer patient was found to exhibit both responses to a newly described tumor antigen.

49. Gansbacher B, Rosenthal FM, Zier K: **Retroviral vector-mediated cytokine-gene transfer into tumor cells.** *Cancer Invest* 1993, **11**: 345–354.

50. Tsai S-CJ, Gansbacher B, Tait L, Miller FR, Heppner GH: **Induction of antitumor immunity by interleukin-2 gene-transduced mouse mammary tumor cells versus transduced mammary stromal fibroblasts.** *J Natl Cancer Inst* 1993, **85**:546–553.

51. Peplinski GR, Tsung K, Meko JB, Norton JA: **Prevention of murine breast cancer by vaccination with tumor cells modified by cytokine-producing recombinant vaccinia viruses.** *Annal Surg Oncol* 1996, **3**:15–23.

52. Zajac P, Schutz A, Oertli D, *et al*: **Enhanced generation of cytotoxic T lymphocytes using recombinant vaccinia virus expressing human tumor-associated antigens and B7 costimulatory molecules.** *Cancer Res* 1998, **58**:4567–4571.

53. Kantor J, Irvine K, Abrams S, *et al*: **Antitumor activity and immune resonses induced by a recombinant carcinoembryonic antigen-vaccinia virus vaccine.** *J Natl Cancer Inst* 1992, **84**:1084–1091.

54. Puisieux I, Odin L, Poujol D, *et al*: **Canarypox virus-mediated interleukin 12 gene transfer into a murine mammary adenocarcinoma induces tumor suppression and long-term antitumoral immunity.** *Hum Gene Ther* 1998, **9**:2481–2492.

55. McAneny D, Ryan CA, Beazley RM, Kaufman HL: **Results of a phase I trial of a recombinant vaccinia virus that expresses carcinoembryonic antigen in patients with advanced colorectal cancer.** *Ann Surg Oncol* 1996, **3**:495–500.

56. Marshall JL, Hawkins MJ, Tsang KY, *et al*: **Phase I study in cancer patients of a replication-defective avipox recombinant vaccine that expresses human carcinoembryonic antigen.** *J Clin Oncol* 1999, **17**:332–337.

57. Rosenberg SA, Zhai Y, Yang JC, *et al*: **Immunizing patients with metastatic melanoma using recombinant adenoviruses encoding MART-1 or gp100 melanoma antigens.** *J Natl Cancer Inst* 1998, **90**:1894–1900.

58. Philip R, Clary B, Brunette E, *et al*: **Gene modification of primary tumor cells for active immunotherapy of human breast and ovarian cancer.** *Clin Cancer Res* 1996, **2**:59–68.

59. Wolff JA, Ludtke JJ, Acsadi G, Williams P, Jani A: **Long-term expression of plasmid DNA and foreign gene expression in mouse muscle.** *Hum Mol Genet* 1992, **1**:363–369.

60. Conry RM, LoBuglio AF, Loechel F, *et al*: **A carcinoembryonic antigen polynucleotide vaccine has *in vivo* antitumor activity.** *Gene Ther* 1995, **2**:59–65.

61. Conry RM, LoBuglio AF, Curiel DT: **Polynucleotide-mediated immunization therapy of cancer.** *Semin Oncol* 1996, **23**:135–147.

62. Amici A, Venanzi FM, Concetti A: **Genetic immunization against neu/erbB2 transgenic breast cancer.** *Cancer Immunol Immunother* 1998, **47**:183–190.

63. Sato Y, Roman M, Tighe H, *et al*: **Immunostimulatory DNA sequences necessary for effective intradermal gene immunization.** *Science* 1996, **273**:352–354.

64. • Ying H, Zaks TZ, Wang RF, *et al*: **Cancer therapy using a self-replicating RNA vaccine.** *Nature Med* 1999, **5**:823–827.
This study shows that replicative RNA vectors are powerful for the induction of immune response. The basis of the immungenicity of this system is investigated.

65. Colmenero P, Liljestrom P, Jondal M: **Induction of P815 tumor immunity by recombinant Semliki Forest virus expressing the P1A gene.** *Gene Ther* 1999, **6**:1728–1733.

66. Tang D, DeVit M, Johnston SA: **Genetic immunization is a simple method for eliciting an immune response.** *Nature* 1992, **356**:152–154.

67. • Tuting T, Gambotto A, Robbins PD, Storkus WJ, DeLeo AB: **Co-delivery of T helper 1-biasing cytokine genes enhances the efficacy of gene gun immunization of mice: studies with the model tumor antigen beta-galactosidase and the BALB/c Meth A p53 tumor-specific antigen.** *Gene Ther* 1999, **6**:629–636.
This paper describes modification of the gene gun approach to favor T-helper 1 response, thereby establishing the utility of this technology for cancer immunotherapy.

68. Banchereau J, Steinman RM: **Dendritic cells and the control of immunity.** *Nature* 1998, **392**:245–252.

69. Hsu FJ, Engleman EG, Levy R, *et al*: **Vaccination of patients with B-cell lymphoma using autologous antigen-pulsed dendritic cells.** *Nature Med* 1996, **2**:52–58.

70. Nestle FO, Alijagic S, Gilliet M, *et al*. **Vaccination of melanoma patients with peptide- or tumor lysate-pulsed dendritic cells.** *Nature Med* 1998, **4**:328–332.

71. Dhodapkar MV, Steinman RM, Sapp M, *et al*: **Rapid generation of broad T-cell immunity in humans after a single injection of mature dendritic cells.** *J Clin Invest* 1999, **104**:173–180.

72. Arthur JF, Butterfield LH, Roth MD, *et al*: **A comparison of gene transfer methods in human dendritic cells.** *Cancer Gene Ther* 1997, **4**:17–25.

73. Brossart P, Goldrath AW, Butz EA, *et al*: **Virus-mediated delivery of antigenic epitopes into dendritic cells as a means to induce CTL.** *J Immunol* 1997, **158**:3270–3276.

74. Song W, Kong HL, Carpenter H,, *et al*: **Dendritic cells genetically modified with an adenovirus vector encoding the cDNA for a model antigen induce protective and therapeutic antitumor immunity.** *J Exp Med* 1997, **186**:1247–1256.

75. Specht JM, Wang G, Do MT, *et al*: **Dendritic cells retrovirally transduced with a model antigen gene are therapeutically effective against established pulmonary metastases.** *J Exp Med* 1997, **186**:1213–1221.

76. Dietz AB, Vuk-Pavlovic S: **High efficiency adenovirus mediated gene transfer to human dendritic cells.** *Blood* 1998, **91**:392–398.

77. • Tillman B, de Gruijl T, Luykx-de Bakker SA, *et al*: **Maturation of dendritic cells accompanies high-efficiency gene transfer by a CD40-targeted adenoviral vector.** *J Immunol* 1999, **162**:6378–6383.
In this study a bispecific antibody was used to target the adenovirus specifically to the DC. Such an approach may eventually permit *in vivo* transduction of DCs.

78. Boczkowski D, Nair SK, Snyder D, Gilboa E: **Dendritic cells pulsed with RNA are potent antigen-presenting cells *in vitro* and *in vivo*.** *J Exp Med* 1996, **184**:465–472.

Author's affiliation: Gene Therapy Center and Division of Hematology Oncology, Department of Medicine, University of Alabama at Birmingham, Birmingham, Alabama, USA

Sponsorship: TVS is supported by the US Army Medical Research and Materiel Command, Department of the Army Award Number DAMD17-1-7243.

Correspondence: Theresa V Strong, Gene Therapy Center and Division of Hematology Oncology, Department of Medicine, University of Alabama at Birmingham, WTI 520, 1824 6th Avenue South, Birmingham, AL 35294-3300, USA. Tel: +1 205 975 9878; fax: +1 205 975 6911; e-mail: theresa.strong@ccc.uab.edu