Evidence of Determinant Spreading in the Antibody Responses to Prostate Cell Surface Antigens in Patients Immunized with Prostate-specific Antigen[1]

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ABSTRACT

Purpose: **Prostate cancer consistently remains a difficult clinical problem. The development of novel therapy strategies for effective control and treatment of prostate cancer is essential. The prostate represents a unique site for immunotherapy, in part because prostate-specific immunity would most probably be without significant long-term sequellae. Antibodies and cell-mediated immunity, induced by either active or passive immunization, represent potential means to specifically target prostate tumor cells.**

Experimental Design: **The serum IgG response to cell surface antigens expressed on LNCAP [prostate-specific antigen (PSA)-positive] and PC-3 (PSA-negative) were analyzed in individuals with advanced disease receiving vaccinia- or fowlpox-expressed PSA (v-PSA or f-PSA, respectively) by flow cytometry.**

Results: **Sera from all seven patients in a Phase I study of v-PSA, collected prior to the third immunization, reacted with both prostate tumor cell lines. The majority of individuals ($n = 12$) in a Phase II trial of v-PSA and f-PSA developed sustainable antibody responses to cell surface antigens on the prostate tumor cell lines. The magnitude and kinetics of these responses were dependent on the immunization schedule. Of importance, the baseline serum of only one of nine patients tested had reactivity with nonprostate tumor cell lines. Sera from three normal males also lacked reactivity with prostate tumor cells.**

Conclusions: **PSA vaccine constructs are immunogenic and induce antibody responses to a multitude of surface antigens on prostate tumor cell lines by epitope or determinant spreading after stimulation of the immune system by PSA immunization.**

INTRODUCTION

Prostate cancer remains a difficult clinical problem, the most diagnosed cancer and the second leading cause of mortality from cancer among American men. The development of novel therapy strategies for effective control and treatment of prostate cancer is essential. Studies have shown that gene-modified tumor vaccines expressing specific cytokines, including interleukin 2, IFNγ (1, 2) and GM-CSF[3] (3), induce T-cell-specific responses that can result in antitumor effects. Highly restricted antigens, including PSA, prostate-specific membrane antigen, and prostatic acid phosphatase, expressed on prostate tumor cells (and normal prostate) have been identified and could serve as targets for vaccines. Vaccination with autologous dendritic cells pulsed with prostate-specific membrane antigen peptides produced CTL and clinical responses in a number of prostate cancer patients with recurrent (4) or hormone-refractory metastatic disease (5). When prostate cancer patients received dendritic cells pulsed with a fusion protein of prostatic acid phosphatase and GM-CSF, all of the patients developed T-cell-proliferative responses, and 52% developed antibody responses to the construct (6). Although it has been shown *in vitro* that allogeneic dendritic cells can stimulate a CTL response (7, 8), the application of antigen-pulsed dendritic cells or gene-transduced tumor cells in humans remains constrained by the requirement to prepare HLA-specific individual vaccines for each recipient.

To circumvent the problem of individually designed vaccines, a number of strategies are being developed to improve the immunogenicity of antigens expressed on prostate cancer cells. Given that the prostate is a unique site in that it is not necessary for maintenance of a healthy life, prostate-specific antigens and associated antigens expressed on normal prostate tissue and on malignant tissue can be targeted. Both IgG and IgM antibody responses have been observed after immunization with globo H hexasaccharide, which is a carbohydrate antigen expressed on the surface of prostate cells (9). These results suggest that it is possible to overcome tolerance and induce an antibody response to an antigen expressed on the surface of normal prostate cells. Protein antigens, including PSA, represent more robust targets for induction of CTL and antibody responses. PSA is expressed almost exclusively on normal and transformed prostatic epithelial cells and represents a candidate for immunotherapy. Studies using different immunization protocols have shown that antibody responses can be induced by vaccination with PSA (10,

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[3] The abbreviations used are: GM-CSF, granulocyte macrophage colony-stimulating factor; PSA, prostate-specific antigen; v-PSA, vaccinia-expressed PSA; f-PSA, fowlpox-expressed PSA; SCC, squamous cell carcinoma; TAA, tumor-associated antigen.

11). When men with advanced disease were immunized with v-PSA, 42% had stable PSA levels for at least 6 months, and 70% of those tested had T-cell-proliferative responses to a PSA peptide (12). Together, these studies suggest that prostate tumor cells can be rendered immunogenic and induce an immune response. However, identification of consistent correlations between induced immune responses and clinical responses have not been forthcoming. Whereas the major focus of active immunotherapy is on induction of T-cell immunity, humoral immune responses also occur and can mediate antitumor effects. Antibodies are the only proven and approved immunotherapy for cancer. Antibodies may contribute directly to tumor destruction by antibody- or complement-dependent cellular cytotoxicity. Alternatively, antibodies may alter cell signaling or alter cell-surface receptor interactions as has been shown for Herceptin and Rituxan, which are being used as immunotherapy (13–18). Antibodies can also be genetically modified for more effective immunotherapy. In the study described here, we detected serum IgG antibodies reactive with prostate cancer cells in patients receiving v-PSA alone (12) or combined with f-PSA.

PATIENTS AND METHODS

Patients. Patients involved in a Phase I study of v-PSA (12) and/or a Phase II study of v-PSA and f-PSA were included in these studies. Patients had a histologically confirmed diagnosis of prostatic adenocarcinoma and evidence of metastatic disease, including any of the following: (*a*) positive lymph nodes and PSA ≥10 ng/ml; (*b*) positive bone scan and PSA ≥10 ng/ml; (*c*) postradical prostatectomy with rising PSA and PSA ≥2 ng/ml; or (*d*) postradiation therapy and PSA ≥10 ng/ml. Patients had not had prior hormonal or chemotherapy for advanced disease. Prior exposure to vaccinia was required. Patients were vaccinated at 4-week intervals unless there was evidence of disease progression or unacceptable toxicity. Plasma was obtained just prior to immunization or 1 month after the final immunization. Patients in the Phase I study of v-PSA (12) received a total of three doses and were followed weekly for the first 2 months and then monthly until 6 months after the final dose. A cohort of patients from the Phase I study of v-PSA were enrolled in the Phase II study at least 6 months after the final v-PSA vaccination. Vaccine-naive patients who fulfilled the eligibility criteria above were also included in the Phase II study. Patients received vaccinations at 4-week intervals and were followed weekly until 28 days after the final dose of vaccine and then monthly for 6 months. Plasma was obtained just prior to and 1 month after each vaccine dose.

Cell Lines. The androgen-sensitive cell line LNCaP and the androgen-independent cell line, PC-3 were used as target cells. LNCaP expresses PSA, and PC-3 does not. Cells were obtained from American Type Culture Collection and maintained as adherent cell cultures in RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 0.1 mM sodium pyruvate, 0.1 mM nonessential amino acids, and 10 μg/ml gentamicin. SCC lines of the head and neck were maintained in a similar manner as specificity controls. The specific cell lines tested included SCC15 and SCC68 and were obtained from James Rheinwald, Brigham and Women's Hospital (Boston, MA; Ref. 19).

Table 1 Reactivity of sera with prostate tumor cell lines

Serum was collected from patients following the third immunization with V-PSA, diluted 1:50, and tested for reactivity with LNCaP or PC-3 prostate tumor cells by flow cytometry. Serum from a normal male was used to determine background fluorescence and to set the marker to 5–7%. Data are from histogram analysis of a representative experiment.

Sera were also tested for reactivity, as described above, with a SCC of the head and neck cell line to confirm prostate specificity.

	Reactivity (% positive)		
Patient	PC-3	LNCap	SCC
D024	31	31	6
D026	46	59	25
D030	26	25	3
D021	19	21	6
D028	41	21	8
D012	39	38	5
D029	31	13	9

Flow Cytometry. Cells were harvested using enzyme-free dissociation buffer (Life Technologies, Inc.) to avoid the loss of trypsin-sensitive proteins. Harvested cells were washed and incubated with sera at a minimal dilution of 1:50, to avoid nonspecific effects, for 30 min on ice. After washing, cells were incubated with FITC-conjugated goat antihuman IgG for 30 min on ice. Cells were washed and fixed with 1% paraformaldehyde prior to acquisition on a FACScan flow cytometer using CellQuest software. Serum from at least one healthy control individual was included as a negative control to determine background fluorescence at 5–7%, and serum from a multiparous individual with anti-HLA reactivity (designated SP22) was used as a positive control.

RESULTS

Sera from seven individuals immunized with v-PSA as described (12) were collected after the third immunization and tested for reactivity with prostate tumor cell lines by flow cytometry. As summarized in Table 1, all seven individuals had IgG antibodies reactive with either PC-3 or LNCaP prostate cancer tumor cell lines. Because PC-3 does not express PSA, this reactivity cannot be solely confined to PSA epitopes. The sera were tested for reactivity with SCC cell lines to determine whether the antibody response was specific to prostate tumor cells and not a consequence of cell culture or nonspecific effects of the vaccine. Six of seven sera did not react with SCC head and neck cell lines. As shown in Table 1, serum from one individual, D026, did react; this serum was excluded from further analysis.

There were distinct differences in the patterns of reactivity of the sera with the cells as shown by the representative histograms in Figs. 1 and 2 . There was a shift in a large population of LNCaP cells with serum from donor D024 (Fig. 1*B*). In contrast, sera from donors D021 (Fig. 1*C*) and D030 (Fig. 1*D*) reacted with a minor population of cells with differing intensities of staining. In contrast, serum from donor D021 reacted with few PC-3 cells (Fig. 2*C*), whereas sera from donors D024 (Fig. 2*B*) and D030 (Fig. 2*D*) reacted with a subpopulation of cells (43% for D024 and 24% for D030). It remains to be determined whether these differences represent a change in

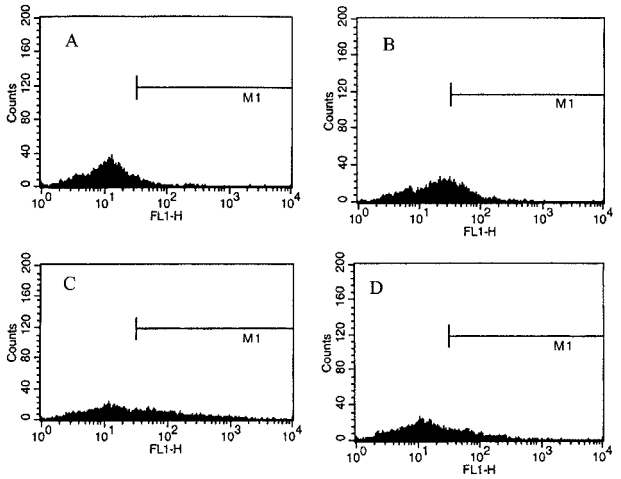

Fig. 1 Histogram analysis of serum reactivity with LNCaP cells. Sera from patients vaccinated with v-PSA were diluted 1:50 and tested for reactivity with LNCaP cells by flow cytometry. Data were analyzed as histograms, using serum from a normal male to determine background fluorescence and to set the marker (*M1*) at 6% in *A*. Results obtained with sera from donors D024 (*B*), D021 (*C*), and D030 (*D*) are also shown. *FL1-H*, log fluorescence intensity.

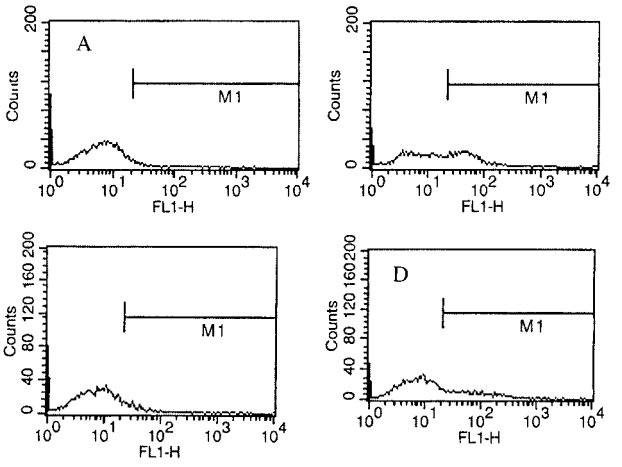

Fig. 2 Histogram analysis of serum reactivity with PC-3 cells. Sera from patients vaccinated with v-PSA were diluted 1:50 and tested for reactivity with PC-3 cells by flow cytometry. Data were analyzed as histograms using serum from a normal male to determine background fluorescence and to set the marker (*M1*) at 5% in *A*. Results obtained with sera from donors D024 (*B*), D021 (*C*), and D030 (*D*) are also shown. *FL1-H*, log fluorescence intensity.

antibody titer, the clonality of the antibody response, or changes in antigenic specificity. It is apparent from the data that the serum antibodies elicited by the PSA vaccines react with epitopes different from PSA and that the differential reactivity to LNCaP and PC-3 suggests multiple unique epitopes.

Baseline sera obtained from three patients (D028, D024, and D030) prior vaccination were available for study. These three prevaccine sera failed to react with prostate (PC-3; as shown in Fig. 3) or SCC control tumor cell lines (data not shown). Hence, antibodies specific to prostate tumor cell lines developed in response to vaccination. We also analyzed baseline sera from nine patients in the Phase II study described below. With the one exception of a single patient with nonspecific tumor cell reactivity of 15%, all of the remaining eight were negative for reactivity with prostate tumor cells prior to vaccination and developed brisk antibody responses after vaccination. Additionally, we tested sera from three normal males and found no reactivity with prostate tumor cells.

Individuals receiving v-PSA have preexisting immunity to vaccinia. This may limit the development of the PSA immune response to multiple vaccinations with this vector. It has been shown that priming the immune system with vaccinia-expressed carcinoembryonic antigen followed by boosting with avipox-encoded carcinoembryonic antigen results in enhanced T-cell responses (20). A Phase II study of a PSA prime/boost strategy using vaccinia- (v-PSA) and avipox-expressed (f-PSA) PSA was initiated. A cohort of patients previously vaccinated with v-PSA in the Phase I study received three doses of f-PSA followed by two additional doses of v-PSA.

As shown in Fig. 4*A*, there was an increase in serum reactivity to prostate tumor cell lines after boosting with f-PSA for four of seven patients. After this initial boost, antibody reactivity decreased to a plateau level seen prior to f-PSA vaccination. A different response pattern of antibody reactivity was observed in the remaining three individuals, as shown in Fig. 4*B*. One patient (D012) maintained consistently low levels of reactivity (12–17%) throughout the study period despite the fact that serum from this individual reacted significantly (36%) during the initial v-PSA immunizations. The reactivity of sera from a second individual declined steadily despite immunization, and there was a sharp decrease in reactivity after f-PSA vaccination in another. For the latter patient (D015), immunization with v-PSA increased serum antibody reactivity.

Vaccine-naive patients with advanced disease were also enrolled in this prime/boost study. Six patients enrolled in the safety cohort and received three immunizations of f-PSA. Patients with responding or stable disease were revaccinated after 6 months, as indicated in Fig. 5. As seen from the first time point taken 6 months after the final f-PSA immunization in the safety study, sera from all individuals reacted with PC-3 cells. Interestingly, after revaccination with f-PSA, a decrease in serum reactivity was observed, which rebounded after the third boost with f-PSA and plateaued with the v-PSA boosts. This is in contrast to the individuals in Fig. 4 who originally received v-PSA and who were crossed over and revaccinated first with f-PSA. For those individuals, there tended to be an increase in serum reactivity after f-PSA. Thus, it can be concluded that the order of initial and subsequent booster immunizations may significantly affect the antibody response to prostate tumor cell lines.

Sera from individuals in the initial v-PSA study have been tested for PSA antibodies by Western blot and peptide ELISA (12). Only one of these individuals developed low-level IgG antibodies to PSA when evaluated in this format. Sera from all individuals collected prior to vaccination and at least two time points post vaccination were tested for reactivity with an irrelevant SCC line. With the exception of the one individual in the Phase I study, all sera failed to react with nonprostate tumor

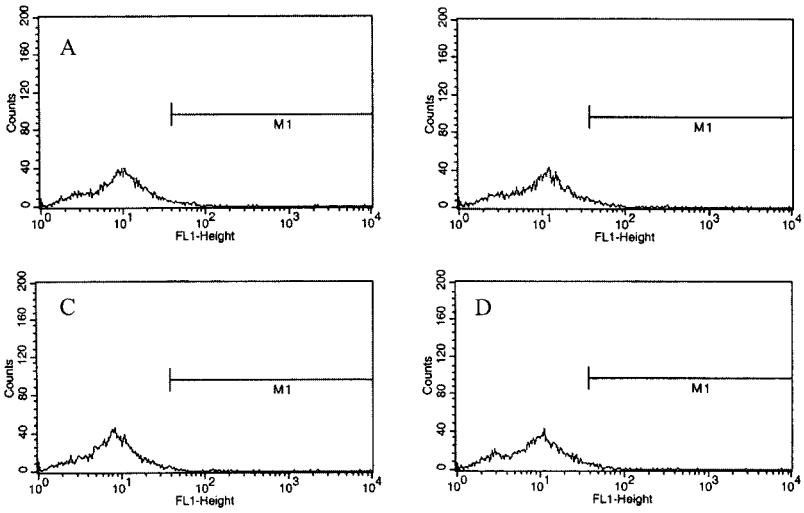

Fig. 3 Failure of baseline sera to react with prostate tumor cells. Sera were collected prior to immunization, diluted 1:50, and tested for reactivity with PC-3 cells by flow cytometry. Data were analyzed as histograms using serum from a normal male to determine background fluorescence and to set the marker (*M1*) at 5% in *A*. Results obtained with sera from donors D028 (*B*), D024 (*C*), and D030 (*D*) are also shown. *FL-1*, log fluorescence intensity.

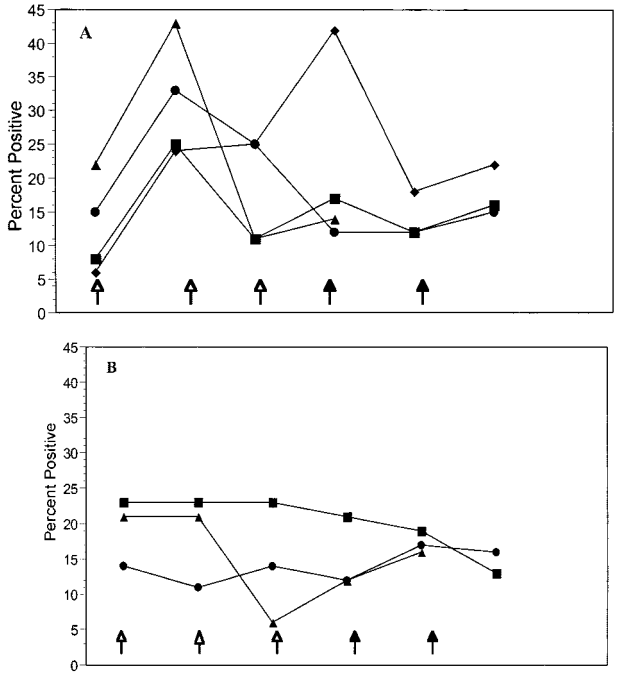

Fig. 4 Reactivity of sera from patients revaccinated with v-PSA and f-PSA with PC-3 cells. Patients previously vaccinated with v-PSA received three immunizations with F-PSA (*open arrows*) followed by two cycles of V-PSA (*filled arrows*), at monthly intervals. Sera were collected just prior to indicated immunization and tested (at 1:50) for reactivity with PC-3 cells by flow cytometry. Patients included in *panel A* are D007 (■), D009 (●), D016 (▲), and D011 (♦). Patients included in *panel B* are D008 (■), D015 (▲), and D012 (●).

cells (data not shown). From these studies, we can conclude that the PSA vaccine constructs induce antibody responses to non-PSA antigens on the surface of prostate tumor cell lines. This conclusion is further supported by the observation that sera react well with PC-3 cells, which are PSA negative. Of more importance, this antibody reactivity is specific to prostate tumor cells and is not evident prior to vaccination.

DISCUSSION

The concept of immunosurveillance was originally proposed by Burnet to describe host resistance to neoplastic disease by T-cell immunity. Since then, it has become clear that although immunosurveillance may be protective against some virally associated tumors, for the most part the immune system fails to protect against malignancy. This is primarily because tumor-specific antigens are not readily identifiable, are poorly immunogenic, or tend to be associated with a poorly immunogenic environment. TAAs have been described for specific tumor types, but these antigens may be normal or altered self-antigens that are not recognized by the immune system or subject to tolerance. Furthermore, TAAs may not be presented to the immune system in an immunogenic manner if MHC-related molecules are absent or altered or may be presented in the context of suppressive factors. For example, presentation of intracellular peptides by MHC class I molecules is important for T-cell responses; however, prostate cancer cells lack MHC class I expression (21). On the other hand, accumulating evidence suggests that the immune system can be educated to recognize TAAs by active immunotherapy in which tolerance is broken or the TAAs are presented in an immunogenic setting by vaccination.

Numerous studies suggest that prostate tumor cells can be rendered immunogenic and induce an immune response (4–11). Antibodies to proteins of 26, 31, and 150 kDa were shown to be induced in some individuals after vaccination with GM-CSF-expressing autologous tumor cells (3). In our study, we detected serum IgG antibodies reactive with native surface antigens on prostate cancer cells in patients receiving v-PSA alone or combined with f-PSA. Although studies are ongoing to identify the antigens recognized by these antibodies, the evidence suggests that the antibody reactivity to prostate cancer cells induced by PSA immunization is independent of a PSA-specific antibody. In particular, with the exception of one serum with low titer, PSA-specific antibodies were not detected in the sera that were tested. Furthermore, sera reacted with PC-3 cells, which are PSA negative. Therefore, we propose that the antiprostate tumor

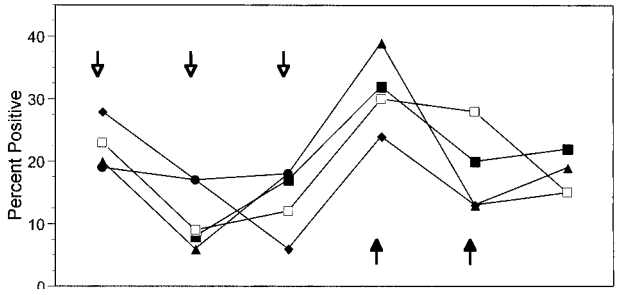

Fig. 5 Reactivity of sera from revaccinated f-PSA safety cohort with PC-3 cells. After a 6-month period, patients in the f-PSA safety cohort (three immunizations) were revaccinated at monthly intervals with f-PSA (*open arrows*) or v-PSA (*filled arrows*). Sera from patients D001 (■), D002 (●), D003 (▲), D004 (♦), and D005 (□) were collected just prior to indicated immunizations and tested (at 1:50) for reactivity with PC-3 cells by flow cytometry. One person was removed from the study.

cell antibody response is explained by epitope or determinant spreading. By determinant spreading, in the process of responding to the immunogen, an immune response is also generated to "bystander" antigens associated with the immunogen in question. Originally it was shown that immunization with a myelin basic protein self-peptide induced immune responses to the entire protein, although the protein was not included in the vaccine (22). Studies have indicated a significant role of determinant spreading in the expanding autoantibody response in murine models of autoimmune disease (23, 24), primate studies (25), and human autoimmunity studies (26). Of importance, not only is there expansion of antibody reactivity to different epitopes on a protein (intramolecular spreading), but also expansion to epitopes on other proteins (intermolecular spreading). Determinant spreading is probably enhanced by local inflammatory responses that contribute to an enhanced immune response to auto- and neoantigens associated with organ-specific tumors. It has been shown that prostate tumor cells secrete a proinflammatory protein in response to stress (27) and that this may occur after PSA immunization and from initial T- and B-cell responses. Determinant spreading would allow for the generation of a broader anti-self-antigen response than is possible by the immunogen alone, which is also the rationale for presenting antigens in the context of vaccinia and fowl pox immune stimulation. Antibodies may independently present antigens to T cells through immune complexes adsorbed by dendritic cells, or B cells may function as antigen-presenting cells for T-cell-mediated immunity.

Humoral immune responses may mediate antitumor effects and enhance cellular immunity. Antibodies may contribute directly to tumor destruction by antibody- or complement-dependent cellular cytotoxicity. Alternatively, antibodies may alter cell signaling or alter cell-surface receptor interactions. Antibodies can also be modified for more effective immunotherapy. For example, heterobifunctional antibodies with dual specificities, one for the target cell and the other for an antigen on killer cells (T cells, natural killer cells), can be useful at directing destruction of tumor cells (28–30). Antibodies can also be used to direct toxins (chemical or biological; Ref. 31). Intracellular expression of antibodies as single chains (scFv) or bifunctional scFv (diabodies) also represents novel therapies because of the increased tissue penetration attributable to their small size (31–34). The conjugation of radioisotopes to antibodies represents potential imaging and therapy options for prostate cancer for a number of reasons. Prostate cancer tends to be relatively radiosensitive and tends to metastasize to areas where antibodies readily infiltrate, including the bone marrow and lymph nodes. The potential of antibodies in cancer imaging and therapy has yet to be realized because of immunogenicity problems caused by xenogeneic antibodies. A significant number of individuals receiving murine monoclonal antibodies, the most readily obtainable antibodies, generate a human-antimouse antibody response. Even with the advent of chimeric and humanized antibodies, there are still problems with immunogenicity and, occasionally, function of the antibodies *in vivo*.

Understanding the humoral immune response to tumor vaccines, especially those that are effective in inducing any clinical or immunological responses, will contribute to the design of more effective immunogens for active immunization. These antibodies may also be of therapeutic value as a component of a vaccine, as imaging agents, or as immunotherapy, particularly for recurrent or hormone-refractory disease. Of importance, we have shown here that immunization with PSA induces spreading of an antibody response to antigen(s) on the surface of prostate tumor cells. These antibodies were specific to prostate tumor cells and not present prior to vaccination. Thus, with appropriate immunogen design, the antibody response can be broadened to include antibodies reactive with self-antigens. Characterization of the antigens recognized by these antibodies and further evaluation of clinical correlates will provide important information on the development of more effective immunotherapeutic regimens.

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REFERENCES

1. Sanda, M. G., Ayyagari, S. R., Jaffee, E. M., Epstein, J. I., Clift, S. L., Cohen, L. K., Dranoff, G., Pardoll, D. M., Mulligan, R. C., and Simons, J. W. Demonstration of a rational strategy for human prostate cancer gene therapy. J. Urol., *151:* 622–628, 1994.

2. Vieweg, J., Rosenthal, F., Bannerji, R., Heston, W., Fair, W., Gansbacher, B., and Gilboa, E. Immunotherapy of prostate cancer in the Dunning rat model: use of cytokine gene modified tumor vaccines. Cancer Res., *54:* 1760–1766, 1994.

3. Simons, J. W., Mikhak, B., Chang, J-F., DeMarzo, A. M., Carducci, M. A., Lim, M., Weber, C. E., Baccala, A. A., Goemann, M. A., Clift, S. M., Ando, D. G., Levitsky, H. I., Cohen, L. K., Sanda, M. G., *et al*. Induction of immunity to prostate cancer antigens: results of a clinical trial of vaccination with irradiated autologous prostate tumor cells engineered to secrete granulocyte-macrophage colony-stimulating factor using *ex vivo* gene transfer. Cancer Res., *59:* 5160–5168, 1999.

4. Murphy, G., Tjoa, B., Simmons, S., Ragde, H., Rogers, M., Elgamal, A., Kenny, G., Troychak, M., Salgaller, M., and Boynton, A. Phase II prostate cancer vaccine trial: report of a study involving 37 patients with disease recurrence following primary treatment. Prostate, *39:* 54–59, 1999.

5. Murphy, G., Tjoa, B., Simmons, S., Jarisch, J., Bowes, V., Ragde, H., Rogers, M., Elgamal, A., Kenny, G., Cobb, O., Ireton, R., Troychak, M.,

Salgaller, M., and Boynton, A. Infusion of dendritic cells pulsed with HLA-A2-specific prostate-specific membrane antigen peptides: a Phase II prostate cancer vaccine trial involving patients with hormone-refractory metastatic disease. Prostate, *38:* 73–78, 1999.

6. Small, E. J., Fratesi, P., Reese, D. M., Strang, G., Laus, R., Peshwa, M. V., and Valone, F. H. Immunotherapy of hormone-refractory prostate cancer with antigen-loaded dendritic cells. J. Clin. Oncol., *18:* 3894–3903, 2000.

7. Nouri-Shirazi, M., Banchereau, J., Bell, D., Burkeholder, S., Kraus, E. T., Davoust, J., and Palucka, K. A. Dendritic cells capture killed tumor cells and present their antigens to elicit tumor-specific immune responses. J. Immunol., *165:* 3797–3803, 2000.

8. Heiser, A., Dahm, P., Yancey, D. R., Maurice, M. A., Boczkowski, D., Nair, S. K., Gilboa, E., and Vieweg, J. Human dendritic cells transfected with RNA encoding prostate-specific antigen stimulate prostate-specific CTL responses *in vitro*. J. Immunol., *164:* 5508–5514, 2000.

9. Slovin, S., Ragupathi, G., Adluris, S., Ungers, G., Terry, K., Kim, S., Spassova, M., Bornmann, W., Fazzari, M., Dantis, L., Olkiewicz, K., Lloyd, K., Livingston, P., Danishefsky, S., and Scher, H. Carbohydrate vaccines in cancer: immunogenicity of a fully synthetic globo H hexasaccharide conjugate in man. Proc. Natl. Acad. Sci. USA, *96:* 5710–5715, 1999.

10. Harris, D. T., Matyas, G. R., Gomella, L. G., Talor, E., Winship, M. D., Spitler, L. E., and Mastrangelo, M. J. Immunologic approaches to the treatment of prostate cancer. Semin. Oncol., *26:* 439–447, 1999.

11. Meidenbauer, N., Harris, D. T., Spitler, L. E., and Whiteside, T. L. Generation of PSA-reactive effector cells after vaccination with a PSA-based vaccine in patients with prostate cancer. Prostate, *43:* 88–100, 2000.

12. Eder, J., Kantoff, P., Rober, K., Xu, G., Bubley, G., Boyden, J., Gritz, L., Mazzara, G., Oh, W., Arlen, P., Tsang, A., Panicali, D., Schlom, J., and Kufe, D. A Phase I trial of a recombinant vaccinia virus expressing prostate-specific antigen in advanced prostate cancer. Clin. Cancer Res., *6:* 1632–1638, 2000.

13. Cragg, M., French, R., and Glennie, M. Signaling antibodies in cancer therapy. Curr. Opin. Immunol., *11:* 541–547, 1999.

14. Kumar, R., Mandal, M., and Vadlamudi, R. New insights into anti-HER-2 receptor monoclonal antibody research. Semin. Oncol., *27:* 84–91, 2000.

15. Yarden, Y., and Sliwkowski, M. Untangling the ErbB signalling network. Nat. Rev. Mol. Cell. Biol., *2:* 127–137, 2001.

16. Pegram, P., and Slamon, D. Biological rationale for HER2/neu (c-erbB2) as a target for monoclonal antibody therapy. Semin. Oncol., *27:* 13–19, 2000.

17. Neve, R., Nielsen, U., Kirpotin, D., Poul, M., Marks, J., and Benz, C. Biological effects of anti-ErbB2 single chain antibodies selected for internalizing function. Biochem. Biophys. Res. Commun., *280:* 274–279, 2001.

18. Shan, D., Ledbetter, J., and Press, O. Signaling events involved in anti-CD20 induced apoptosis of malignant human B cells. Cancer Immunol. Immunother., *48:* 673–683, 2000.

19. Rheinwald, J., and Beckett, M. Tumorigenic keratinocyte lines requiring anchorage and fibroblast support cultured from human squamous cell carcinoma. Cancer Res., *41:* 1657–1663, 1981.

20. Marshall, J. L., Hoyer, R. J., Toomey, M. A., Faraguna, K., Chang, P., Richmond, E., Pedicano, J. E., Gehan, E., Peck, R. A., Arlen, P., Tsang, K. Y., and Schlom, J. Phase I study in advanced cancer patients of a diversified prime-and-boost vaccination protocol using recombinant vaccinia virus and recombinant nonreplicating avipox virus to elicit anti-carcinoembryonic antigen immune responses. J. Clin. Oncol., *18:* 3964–3973, 2000.

21. Blades, R., Keating, P., McWilliam, L., George, N., and Stern, P. Loss of HLA class I expression in prostate cancer. Implications for immunotherapy. Urology, *46:* 681–685, 1995.

22. Lehmann, P., Forsthuber, T., Miller, A., and Sercarz, E. Spreading of T-cell autoimmunity to cryptic determinants of an autoantigen. Nature (Lond.), *358:* 155–157, 1992.

23. Farris, A., Brown, L., Reynolds, P., Harley, J., James, J., Scofield, R., McCluskey, J., and Gordon, T. Induction of autoimmunity by multivalent immunodominant and subdominant T cell determinants of La(SS-B). J. Immunol., *162:* 3079–3087, 1999.

24. Tian, J., Lehmann, P., and Kaufman, D. Determinant spreading of T helper cell 2 (Th2) responses to pancreatic islet autoantigens. J. Exp. Med., *186:* 2039–2043, 1997.

25. McFarland, H., Lobito, A., Johnson, M., Nyswaner, J., Frank, J., Palardy, G., Tresser, N., Genain, C., Mueller, J., Matis, L., and Lenardo, M. Determinant spreading associated with demyelination in a nonhuman primate model of multiple sclerosis. J. Immunol., *162:* 2384–2390, 1999.

26. Tuohy, V., Yu, M., Yin, L., Kawczak, J., and Kinkel, R. Spontaneous regression of primary autoreactivity during chronic progression of experimental autoimmune encephalomyelitis and multiple sclerosis. J. Exp. Med., *189:* 1033–1042, 1999.

27. Barnett, G., Jakobsen, A-M., Tas, M., Rice, K., Carmichael, J., and Murray, J. Prostate adenocarcinoma cells release the novel proinflammatory polypeptide EMAP-II in response to stress. Cancer Res., *60:* 2850–2857, 2000.

28. Cochlovius, B., Kipriyanov, S. M., Stassar, M. J. J. G., Christ, O., Schuhmacher, J., Straub, G., Moldenhauer, G., and Little, M. Treatment of human B cell lymphoma xenografts with a CD3×CD19 diabody and T cells. J. Immunol., *165:* 888–895, 2000.

29. Scott, C., Blattler, W., Lambert, J., Kalish, R., Morimoto, C., and Schlossman, S. Requirements for the construction of antibody heterodimers for the direction of lysis of tumors by human T cells. J. Clin. Investig., *81:* 1427–1433, 1988.

30. Segal, D., and Wunderlich, J. Targeting of cytotoxic cells with heterocrosslinked antibodies. Cancer Investig., *6:* 83–92, 1988.

31. Yuan, F., Baxter, L., and Jain, R. Pharmacokinetic analysis of two-step approaches using bifunctional and enzyme-conjugated antibodies. Cancer Res., *51:* 3119–3130, 1991.

32. Hu, Z., and Garen, A. Intratumoral injection of adenoviral vectors encoding tumor-targeted immunoconjugates for cancer immunotherapy. Proc. Natl. Acad. Sci. USA, *97:* 9221–9225, 2000.

33. Nielsen, U. B., Adams, G. P., Weiner, L. M., and Marks, J. D. Targeting of bivalent anti-erbB2 diabody antibody fragments to tumor cells is independent of the intrinsic antibody affinity. Cancer Res., *60:* 6434–6440, 2000.

34. Tse, E., and Rabbitts, T. H. Intracellular antibody-caspase-mediated cell killing: an approach for application in cancer therapy. Proc. Natl. Acad. Sci. USA, *97:* 12266–12271, 2000.

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