Anti-PSCA mAbs inhibit tumor growth and metastasis formation and prolong the survival of mice bearing human prostate cancer xenografts

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Prostate stem-cell antigen (PSCA) is a cell-surface antigen expressed in normal prostate and overexpressed in prostate cancer tissues. PSCA expression is detected in over 80% of patients with local disease, and elevated levels of PSCA are correlated with increased tumor stage, grade, and androgen independence, including high expression in bone metastases. We evaluated the therapeutic efficacy of anti-PSCA mAbs in human prostate cancer xenograft mouse models by using the androgen-dependent LAPC-9 xenograft and the androgen-independent recombinant cell line PC3-PSCA. Two different anti-PSCA mAbs, 1G8 (IgG1κ) and 3C5 (IgG2aκ), inhibited formation of s.c. and orthotopic xenograft tumors in a dose-dependent manner. Furthermore, administration of anti-PSCA mAbs led to retardation of established orthotopic tumor growth and inhibition of metastasis to distant sites, resulting in a significant prolongation in the survival of tumor-bearing mice. These studies suggest PSCA as an attractive target for immunotherapy and demonstrate the therapeutic potential of anti-PSCA mAbs for the treatment of local and metastatic prostate cancer.

Prostate cancer is the most commonly diagnosed cancer and is the second leading cause of cancer-related deaths in American males (1). Despite curative therapies for localized disease such as radical prostatectomy, radiation therapy, and cryotherapy, approximately one-third of treated patients will relapse (2, 3). Androgen-ablation therapy is effective initially in advanced metastatic disease, but in most cases androgen-independent tumors develop for which there is no available effective therapy (3). Recently, mAb therapy has proven efficacious in clinical cancer treatment, including an anti-CD20 mAb (Rituxan) for B cell lymphoma (4) and an anti-Her2/neu mAb (Herceptin) for metastatic breast cancer (5). Development of antibody therapeutics for prostate cancer is limited by the paucity of cell-surface antigens expressed at a high frequency and at a significant level in prostate-cancer patients and with a restricted expression pattern in normal tissues. At present, antibodies to only one such target antigen, prostate-specific membrane antigen, are being developed toward clinical trials (6, 7).

Recently, we reported the identification and characterization of prostate stem-cell antigen (PSCA), a cell-surface antigen that is predominantly prostate specific and expressed in the majority of prostate-cancer patients (8–10). PSCA is a glycosylphosphatidylinositol-anchored 123-aa protein related to the Ly-6 family of cell-surface proteins. *In situ* hybridization and immunohistochemical (IHC) analysis demonstrated PSCA expression in over 80% of local disease specimens and in all bone metastatic lesions examined (8, 9). Elevated PSCA expression was shown to correlate with increased tumor stage, grade, and progression to androgen independence (9). The significant cell-surface expression of PSCA in localized and advanced disease, together with its restrictive expression in normal tissues (9), makes PSCA a potential target for immunotherapy. Recent studies have indicated PSCA to be a target for T cell-based immunotherapy (11); however, PSCA antibody therapy has not been investigated yet.

Here we examined the anti-tumor efficacy of two anti-PSCA mAbs with different isotypes and affinities: 1G8 (IgG1κ; K_D = 10^{-9} M) and 3C5 (IgG2aκ; K_D = 4.3×10^{-8} M). Antibody efficacy on tumor growth and metastasis formation was studied primarily in a mouse orthotopic prostate-cancer xenograft model. In this report we demonstrate that anti-PSCA mAbs are able to inhibit formation of both the androgen-dependent LAPC-9 (12) and the androgen-independent PC3-PSCA (13) tumor xenografts. Anti-PSCA mAbs also retarded the growth of established orthotopic tumors significantly and prolonged survival of tumor-bearing mice. Strikingly, antibody treatment resulted in nearly a complete inhibition of lung metastasis formation in tumor-bearing mice. These results indicate the potential utility of anti-PSCA mAbs in the treatment of local and advanced stages of prostate cancer.

Materials and Methods

Prostate Cancer Xenografts and Cell Lines. The LAPC-9 xenograft, which expresses a wild-type androgen receptor and produces prostate-specific antigen (PSA), was passaged in 6- to 8-week-old male ICR-severe combined immunodeficient (SCID) mice (Taconic Farms) by s.c. trocar implant (12). Single-cell suspensions of LAPC-9 tumor cells were prepared as described (12). The prostate carcinoma cell line PC3 (American Type Culture Collection) was maintained in DMEM supplemented with L-glutamine and 10% (vol/vol) FBS. A PC3-PSCA cell population was generated by retroviral gene transfer as described (14). Expression of PSCA on the cell surface was determined by staining 1×10^6 cells at 4°C for 30 min with 1 μg of either the 1G8 or 3C5 mAbs and 1 μg of IgG1 or IgG2a isotype control antibodies, respectively. Anti-PSCA staining was detected by using an FITC-conjugated goat anti-mouse antibody (Southern Biotechnology Associates) followed by analysis on a Coulter Epics-XL flow cytometer.

Purification and Characterization of Anti-PSCA mAbs. The 1G8 (IgG1κ) and 3C5 (IgG2aκ) anti-PSCA hybridomas, derived from mice immunized with a glutathione *S*-transferase–PSCA fusion protein (9), were purified from ascites. Affinity measurements were performed by surface plasmon resonance technology using BIAcore 2000 (Amersham Pharmacia) with purified mAbs and purified secPSCA immobilized at low density (30 RU). The association- and dissociation-rate constants were determined by using CLAMP software (15).

Abbreviations: PSCA, prostate stem-cell antigen; IHC, immunohistochemical; PSA, prostate-specific antigen; SCID, severe combined immunodeficient; STEAP, six-transmembrane epithelial antigen of the prostate.

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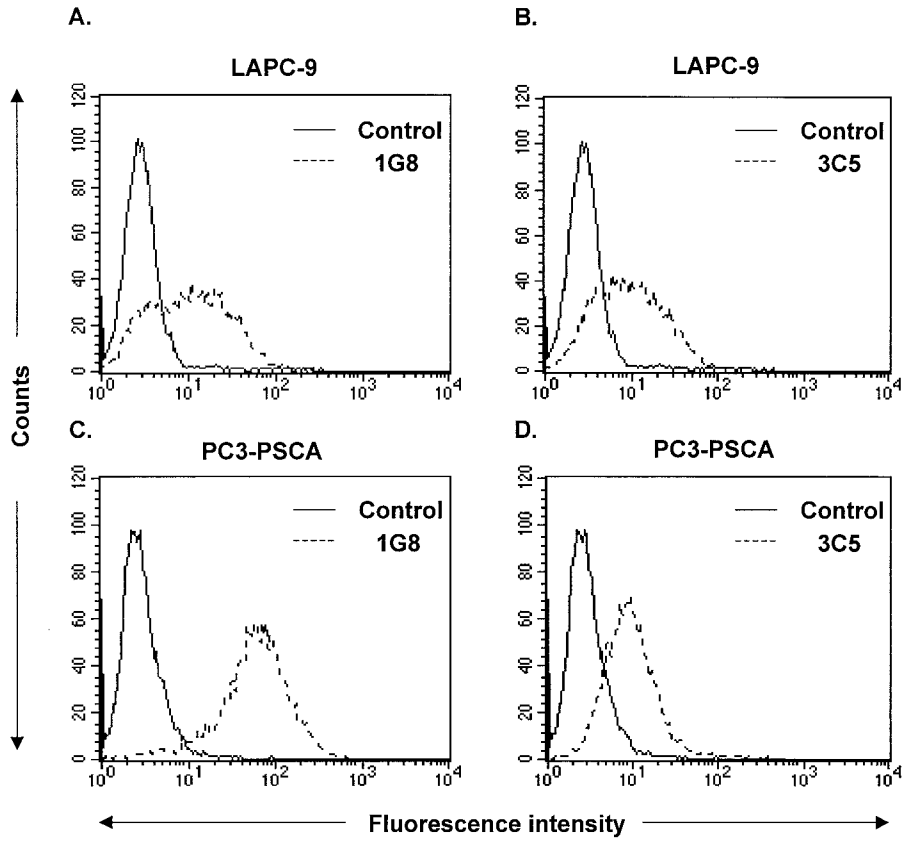

Fig. 1. Detection of cell-surface PSCA expression by anti-PSCA mAbs using flow cytometry. (*A* and *B*) LAPC-9 cells were stained with either 1G8 (*A*) or 3C5 (*B*) mAbs (dotted line) and the corresponding isotype control antibodies (solid line) IgG1 (*A*) or IgG2a (*B*). (*C* and *D*) PC3-PSCA cells were stained with either 1G8 (*C*) or 3C5 (*D*) mAbs (dotted line). As a control, PC3 cells (solid line) were stained with 1G8 (*C*) or 3C5 (*D*).

Xenograft Mouse Models. S.c. tumors were generated by injection of 1×10^6 LAPC-9, PC3, or PC3-PSCA cells mixed at a 1:1 dilution with Matrigel (Collaborative Research) in the right flank of male SCID mice. To test antibody efficacy on tumor formation, i.p. antibody injections started on the same day as tumor-cell injections. As a control, mice were injected with either purified mouse IgG (ICN) or PBS. In preliminary studies, we found no difference between mouse IgG or PBS on tumor growth. Tumor sizes were determined by vernier caliper measurements, and the tumor volume was calculated as length $\times$ width $\times$ height. Mice with s.c. tumors greater than 1.5 cm in diameter were killed in accordance with University of California Los Angeles Animal Rights Committee guidelines. PSA levels were determined by using a PSA ELISA kit (Anogen, Mississauga, Ontario). Circulating levels of 3C5 or 1G8 mAbs were determined by a capture ELISA kit (Bethyl Laboratories, Montgomery, TX). Antibody half-life in SCID mice was determined to be 14–15 days.

Orthotopic injections were performed under anesthesia by using ketamine/xylazine. An incision was made through the abdominal muscles to expose the bladder and seminal vesicles, which then were delivered through the incision to expose the dorsal prostate. LAPC-9 cells (5×10^5) mixed with Matrigel were injected into each dorsal lobe in a 10-μl volume. To monitor tumor growth, mice were bled on a weekly basis for determination of PSA levels. Based on the PSA levels, the mice were segregated into groups for the appropriate treatments. To test the effect of anti-PSCA mAbs on established orthotopic tumors, i.p. antibody injections started when PSA levels reached 2–80 ng/ml.

IHC Analysis of Tissue Specimens. IHC analysis was performed on 4-μm formalin-fixed paraffin-embedded prostate and lung tissues derived from mice bearing orthotopic LAPC-9 tumors. Then 50 serial sections were cut from each lung, and every 10th section was stained for expression of the prostate-specific marker six-transmembrane epithelial antigen of the prostate (STEAP) by using an anti-STEAP polyclonal antibody (14). Micrometastases consisting of at least two visible cells in a crosssectional view were scored as positive.

Statistical Analysis. Mean tumor volumes and PSA levels were compared between groups by using a Mann–Whitney *U* test. *P* values <0.05 were considered significant statistically. Mouse survival was analyzed by using a log rank test. *P* values <0.05 were considered significant statistically.

Results and Discussion

Two anti-PSCA mAbs, 1G8 (IgG1κ) and 3C5 (IgG2aκ), were analyzed for anti-tumor activity on the androgen-dependent LAPC-9 xenograft and androgen-independent PC3 cell line engineered to express PSCA (PC3-PSCA), both derived from bone metastases from patients with advanced prostate cancer (12, 13). Flow-cytometric analysis confirmed the ability of anti-PSCA mAbs to detect expression of native PSCA protein on the surface of LAPC-9 and PC3-PSCA cells (Fig. 1). Antibody binding was shown to lead to antigen internalization (data not shown). The 1G8 and 3C5 mAbs were shown previously to recognize the middle portion (amino acids 46–85) and the amino-terminal portion (amino acids 21–50) of the PSCA extracellular domain, respectively (9). Affinities of 1G8 and 3C5 mAbs were determined to be 1×10^{-9} M ($K_{on} = 1.68 \times 10^5$; K_{off}

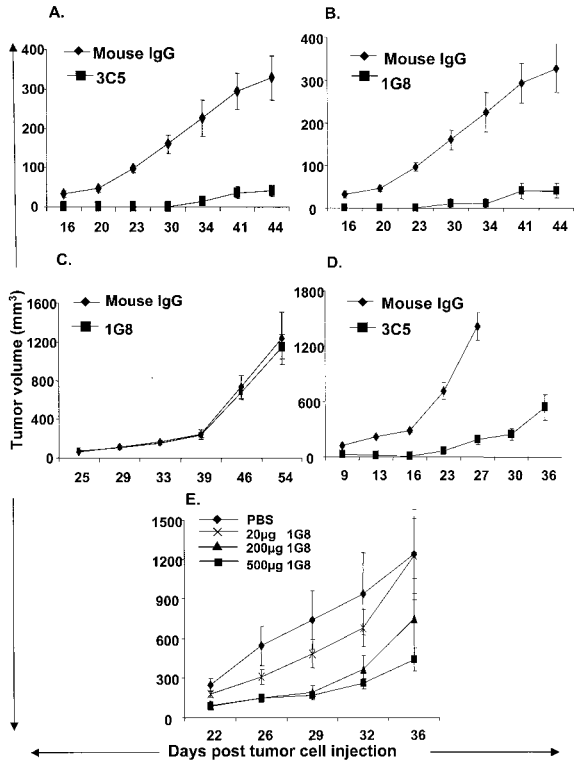

Fig. 2. Inhibition of s.c. tumor formation by anti-PSCA mAbs. (*A–E*). Male SCID mice were injected s.c. with 1×10^6 PC3-PSCA (*A* and *B*), PC3 (*C*), or LAPC-9 (*D* and *E*) cells in conjunction with i.p injection of either 200 μg of 3C5 (*A* and *D*) or 1G8 (*B* and *C*) mAb (■) or 200 μg control mouse IgG (*A–D*, ◆). Antibody doses administered in *E* were either 20 (×), 200 (▲), or 500 μg (■), and PBS (◆) was used as a control. All treatments were carried out three times a week for 2 weeks (six injections total) on eight mice in each group. The data are presented as mean tumor volume (mm^3) ± SEM.

$= 1.69 \times 10^{-4}$) and 4.3×10^{-8} M ($K_{on} = 8.3 \times 10^3$; $K_{off} = 3.58 \times 10^{-4}$), respectively, by solid-phase measurements using purified PSCA extracellular domain as the antigen.

Anti-PSCA mAbs Inhibit Formation of PSCA-Expressing Prostate-Cancer Tumors. To determine whether the anti-PSCA mAbs could inhibit formation of PSCA-expressing tumors *in vivo*, we performed a series of experiments by using 1G8 and 3C5 mAbs with both LAPC-9 and PC3-PSCA xenograft tumors. In the first series of experiments, tumor cells were injected s.c. in the flanks of male SCID mice in conjunction with i.p. injection of 200 μg of 1G8 or 3C5 mAbs or control mouse IgG. Antibody was administered three times per week for two consecutive weeks. Treatment with either 1G8 or 3C5 mAb inhibited formation of PC3-PSCA tumors compared with mice treated with control antibody (Fig. 2 *A* and *B*). Tumor growth was suppressed completely in both the 1G8- and 3C5-treated groups ($P = 0.002$ and $P < 0.001$, respectively) until 14 days after the last antibody injection, at which point tumors grew but at a significantly reduced rate. At day 44 (28 days after last antibody injection), tumor sizes were approximately 8-fold lower in both the 1G8- and 3C5-treated groups (41 mm^3) as compared with the control group (328 mm^3). The 1G8 mAb had no effect on the formation of tumors from parental PC3 cells that do not express PSCA (Fig. 2*C*), demonstrating that the effect on tumor growth results from specific binding to PSCA.

Inhibition of s.c. tumor formation also was observed by antibody treatment of LAPC-9 tumors. LAPC-9 xenografts, which were propagated *in vivo* since their establishment from

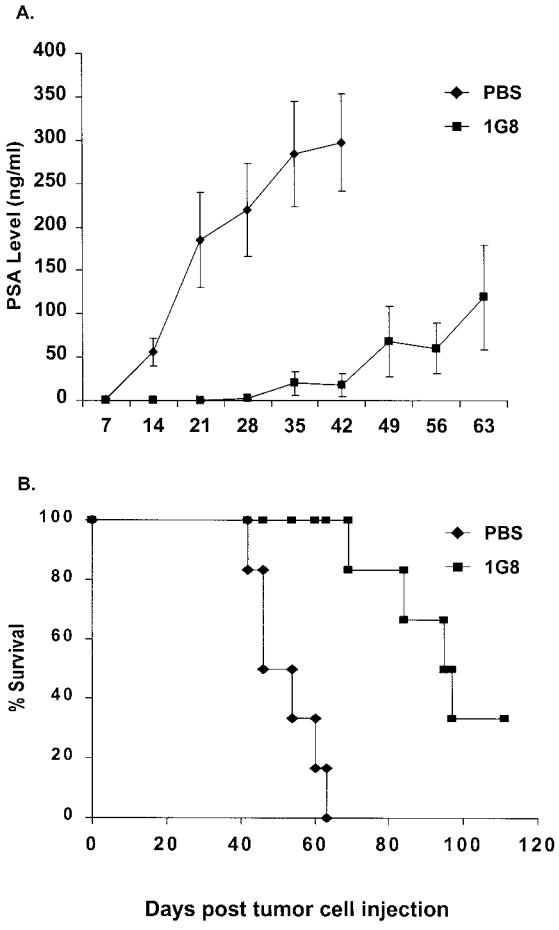

Fig. 3. Inhibition of orthotopic tumor formation by anti-PSCA mAb. LAPC-9 (5×10^5) cells were injected into each dorsal lobe of the prostate in male SCID mice. After 2 days, mice ($n = 6$) were injected i.p. with 200 μg of 1G8 (■) or PBS (◆) three times per week for 2 weeks. (*A*) Tumor growth was monitored by measuring PSA levels on the indicated days. Each data point represents the mean serum PSA levels (± SEM) for each group of six mice. (*B*) Significant prolongation of survival of mice treated with 1G8 (■) compared with mice treated with PBS (◆). The percentage of survival is shown for each group at the indicated time points.

patient-derived bone metastasis, exhibit more aggressive growth than PC3-PSCA (Fig. 2 *D* and *E*). Administration of 3C5 to LAPC-9-injected mice (six injections of 200 μg) resulted in complete inhibition of tumor formation until day 16, as compared with control mice, with a significant reduction ($P < 0.001$) in tumor-growth rate afterward (Fig. 2*D*). At day 27, 11 days after the last antibody injection, tumors in the 3C5-treated mice reached a mean size of 190 mm^3, whereas the mice in the control group exhibited a mean size of 1,400 mm^3 and had to be killed. At day 26, the PSA levels in 3C5-treated mice were 2 ng/ml as compared with 80 ng/ml in the control group, providing further evidence for the antibody effect on tumor formation. Initiation of tumor growth correlated with 2-fold decrease in circulating 3C5 antibody levels (data not shown).

Antibody dose-response experiments were carried out by injecting LAPC-9 cells into mice concomitantly with doses of 20, 200, or 500 μg 1G8 mAb or PBS as a control. Antibody treatment was performed three times per week for two consecutive weeks and resulted in inhibition of LAPC-9 tumor growth in a dose-dependent manner (Fig. 2*E*). Both the 200- and 500-μg doses demonstrated equivalent and significant ($P < 0.01$) inhibition of tumor growth up to day 29, 17 days after the last antibody

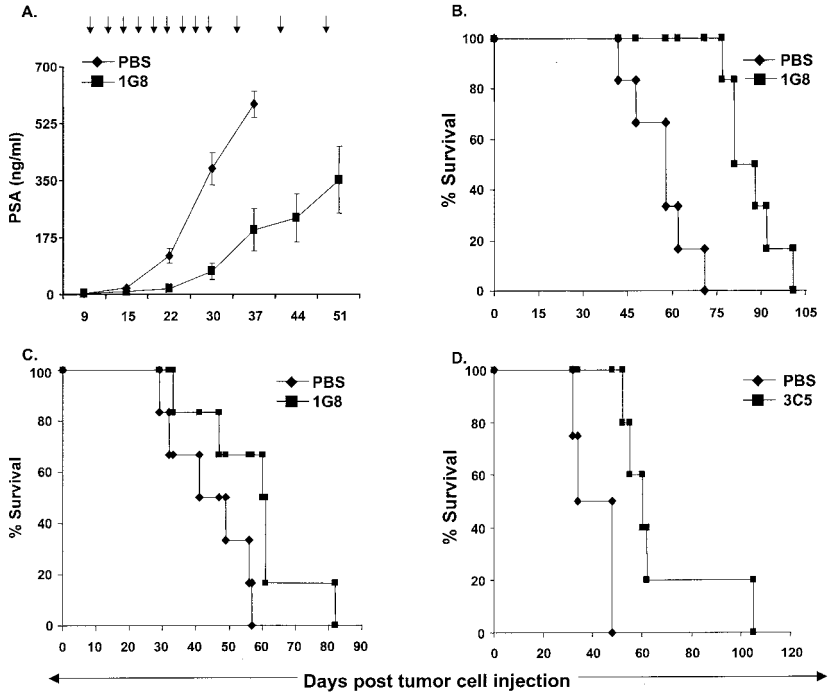

Fig. 4. Growth retardation of established LAPC-9 orthotopic tumors and prolongation of survival of tumor-bearing mice by anti-PSCA mAbs. (*A*) Mice ($n = 6$) with PSA levels of 2–3 ng/ml (day 9) were treated with either PBS (◆) or 2 mg 1G8 (■) three times for 1 week followed by nine injections of 1 mg during the next 3 weeks, as indicated by arrows. Tumor growth was monitored by measuring serum PSA levels, and each data point represents the mean PSA level (± SEM). (*B*) Significant prolongation of survival of 1G8-treated mice (■) in *A* compared with PBS control mice (◆). The percentage of survival is shown for each group at the indicated time points. (*C*) Mice ($n = 5$) with PSA levels of 64–78 ng/ml (day 13) were given 10 injections of 1 mg of 1G8 for 4 weeks. The percentage of survival is shown for each group at the indicated time points. (*D*) Mice ($n = 6$) with PSA levels 14–16 ng/ml (day 7) were injected with 2 mg of 3C5 three times for 1 week, followed by seven injections of 1 mg 3C5 mAb for the next 3 weeks. The percentage of survival is shown for each group at the indicated time points.

injection. Increased tumor volumes were detected in those mice at day 32, at which point a difference in efficacy between the 200- and 500-μg doses became apparent. Minimal inhibition of tumor growth was detected in the group of mice treated with 20 μg 1G8 mAb. The effect of mAb on tumor volumes was corroborated by a dose-dependent reduction in PSA levels detected in 1G8-treated mice (data not shown).

We next tested the effect of anti-PSCA mAbs on tumor formation by using the LAPC-9 orthotopic model. As compared with the s.c. model for tumor growth, the orthotopic model, which requires injection of tumor cells directly in the mouse prostate, results in local tumor growth, development of metastasis in distal sites, deterioration of mouse health, and subsequent death (16, 17). These features make the orthotopic model more representative of human disease progression and allowed us to follow the therapeutic effect of mAbs on clinically relevant end points. LAPC-9 tumor cells were injected into the mouse prostate, and 2 days later the mice were segregated into two groups and treated with either 200 μg of 1G8 mAb or PBS three times per week for two weeks. Mice were monitored weekly for circulating PSA levels as an indicator of tumor growth. In the PBS control group, PSA levels rose steadily to reach mean PSA levels of over 200 ng/ml by day 28 (Fig. 3*A*). No detectable PSA levels were observed in the 1G8-treated mice up to day 28, 12 days after the last antibody injection, suggesting a complete inhibition of LAPC-9 tumor growth. PSA levels started to be detectable in the serum of 1G8-treated mice 19 days after the last antibody injection, likely as a result of the significant drop in 1G8 serum concentration (over 3-fold reduction from day-16 levels, data not shown). At all time points, PSA levels in the 1G8-treated mice were significantly lower as compared with the PBS-control mice ($P < 0.002$). Survival of 1G8-treated mice was also prolonged significantly ($P = 0.0005$). All untreated mice died within 42–63 days, whereas 50% of 1G8-treated mice were still alive by day 95 (Fig. 3*B*). Together, these results demonstrate the ability of anti-PSCA mAbs to inhibit tumor formation in both s.c. and orthotopic tumor models. In both tumor models, initiation of tumor growth paralleled a significant drop in the concentration of serum anti-PSCA antibodies. Animals treated with anti-PSCA mAbs showed a significantly reduced tumor-growth rate as compared with untreated mice.

Anti-PSCA mAbs Retard the Growth of Established Orthotopic Tumors and Prolong Survival of Tumor-Bearing Mice. Orthotopic injection of LAPC-9 cells results in aggressive local tumor growth and metastasis, leading to deteriorated health and eventual death within 6–7 weeks (Fig. 3). This feature allowed us to study the effect of the anti-PSCA mAbs not only on tumor growth but also the health and survival of mice bearing orthotopic LAPC-9 tumors. Initially, we studied the effect of the 1G8 mAb in mice with low established tumor burden (PSA levels 2–3 ng/ml). Mice were injected three times with 2 mg of 1G8 mAb during the first week, followed by nine injections of 1 mg over a 4-week period. A control group was injected with PBS. Tumor growth was monitored weekly by measuring serum PSA levels in both groups. Treatment of the LAPC-9 tumor-bearing mice with 1G8 mAb resulted in highly reduced PSA levels as compared with the control group, demonstrating significant ($P = 0.002$) inhibition of tumor growth (Fig. 4*A*). Mean PSA levels in the 1G8-treated mice were 7-, 5-, and 3-fold lower at days 22, 30, and 37, respectively, than in the PBS-control group. At day 51, the mean PSA level in the 1G8-treated mice was 350 ng/ml, which was equivalent approximately to levels in PBS-treated mice at day 30, indicating retardation in tumor growth of approximately 20 days.

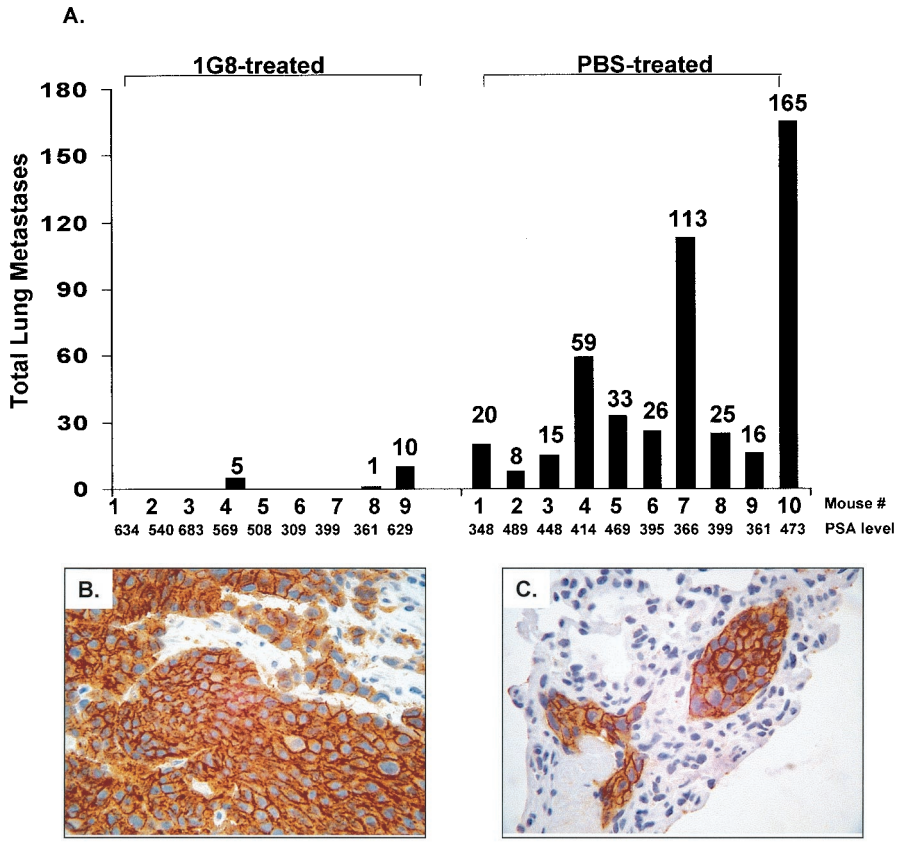

Fig. 5. Anti-PSCA mAb inhibit lung metastasis in mice bearing large orthotopic prostate tumors. Mice with established orthotopic LAPC-9 tumors were treated i.p. with either PBS (control) or 1G8 mAb (1 mg) nine times for 3 weeks. Mice were killed at different times when large prostate tumors were established (PSA >300 ng/ml, see indicated values for each individual mouse). Prostate and lungs were harvested from PBS-treated ($n = 10$) and 1G8-treated ($n = 9$) and processed for IHC analysis by using anti-STEAP antibodies. Total number of lung metastases in five lung sections for each mouse analyzed is shown (*A*). No metastases were detected in 1G8-treated mice 1, 2, 3, 5, 6, and 7. The photomicrographs demonstrate representative staining of local orthotopic tumors (*B*) and lung micrometastases (*C*).

The effect of 1G8 mAb on tumor growth led to a significant prolongation ($P = 0.004$) of survival of the mice. In the 1G8-treated group, the median survival was 89 days (range 77–101 days) vs. 56.5 days (range 42–71 days) in the PBS-treated mice (Fig. 4*B*), representing a median survival increase of 32.5 days.

We then studied the effect of the 1G8 mAb on mice with a higher tumor burden (PSA levels 64–78 ng/ml). Treatment was comprised of 10 injections of 1 mg 1G8 mAb or PBS over a 4-week period. Survival again was prolonged significantly ($P = 0.002$) in the 1G8-treated group, with a median survival of 78.5 days (range 52–105 days) vs. 40 days (range 32–48 days) in the PBS-treated group (Fig. 4*C*), representing a median survival increase of 38.5 days. Significant prolongation of survival was detected also by treatment of established orthotopic LAPC-9 tumors with 3C5 mAb ($P = 0.0005$), leading to an increase in median survival by approximately 15 days compared with control mice (Fig. 4*D*). In all experiments, antibody treatment also showed a prominent effect on the health of the mice. At 5–6 weeks after tumor-cell injection, mice in the control group appeared sickly and cachectic, characterized by sluggishness, an unkempt appearance, and scruffy fur especially around the eyes (data not shown), followed by mortality starting at 5 weeks (Fig. 4). At the same time point, antibody-treated mice appeared healthy and active and maintained normal grooming behavior (data not shown). The health of antibody-treated mice deteriorated at a significantly later time than the control mice, which correlated with their prolonged survival (data not shown).

Anti-PSCA mAbs Prevent Formation of Lung Metastasis from LAPC-9 Orthotopic Tumors. A major advantage of the orthotopic prostate-cancer model is the ability to study the development of metastases. Formation of metastasis in mice bearing established orthotopic tumors was studied by IHC analysis on lung sections using an antibody against a prostate-specific cell-surface protein STEAP expressed at high levels in LAPC-9 xenografts (14). Mice bearing established orthotopic LAPC-9 tumors were administered 11 injections of either 1 mg 1G8 mAb or PBS over a 4-week period. Mice in both groups were allowed to establish a high tumor burden (PSA levels greater than 300 ng/ml), to ensure a high frequency of metastasis formation in mouse lungs. Mice then were killed and their prostate and lungs were analyzed for the presence of LAPC-9 cells by anti-STEAP IHC analysis. Large local prostate tumors expressing STEAP were detected in mice from both groups (Fig. 5*B*). Metastases were detected readily in all lungs analyzed (8–165 per lung) from the 10 PBS-treated mice analyzed (Fig. 5 *A* and *C*). In contrast, no micrometastases were detected in the lungs of six of the nine 1G8-treated mice, and only a few micrometastases were scored in the lungs of the other three mice (Fig. 5*A*). These results demonstrate that anti-PSCA mAb significantly inhibits ($P < 0.0001$) metastasis formation in mice bearing large orthotopic tumor burden (PSA levels 300–650 ng/ml). These striking findings raise the possibility that immunotherapy targeted to PSCA may prevent metastasis formation effectively in patients with established tumors before or after prostatectomy.

Our studies demonstrate a broad anti-tumor efficacy of anti-

PSCA antibodies on initiation and progression of prostate cancer in xenograft mouse models. Anti-PSCA antibodies inhibited tumor formation of both androgen-dependent and androgen-independent tumors as well as retarded the growth of already established tumors significantly and prolonged the survival of treated mice. Moreover, anti-PSCA mAbs demonstrated a dramatic inhibitory effect on the spread of local prostate tumor to distal sites, even in the presence of a large tumor burden. Thus, use of the orthotopic mouse models allowed us to demonstrate significant efficacy of anti-PSCA mAbs on major clinically relevant end points/PSA levels (tumor growth), prolongation of survival, and health.

Anti-prostate tumor activity was demonstrated previously for antibodies directed against the Her-2/Neu (18) and the epidermal growth-factor receptor (EGFR; ref. 19) antigens. Unlike the results obtained with anti-PSCA antibodies, the anti-HER-2/Neu mAb Herceptin affected the growth of androgen-dependent but not androgen-independent xenografts, indicating the necessity for signaling through the androgen receptor for effective Herceptin response (18). The anti-EGFR mAb, C225, significantly inhibited the growth of s.c.-established tumors derived from androgen-independent DU145 and PC3 cells (19).

The mechanism by which the anti-PSCA mAbs exert their effect on the growth of prostate-cancer cells is unknown. Multiple mechanisms have been proposed for the ability of antitumor antibodies to mediate their effect *in vivo*, including programmed cell death, differentiation of tumor cells, or modulation of angiogenesis factors (20–23). An alternative mechanism involves disruption of PSCA-mediated cell–cell or cell–matrix interaction that is critical for local tumor growth and spread to distal sites. Such a mechanism has been implicated for another glycosylphosphatidylinositol-linked Ly6-family member tumor antigen E48, which was found to mediate cell–cell interaction in squamous cell carcinomas (24). In addition, the involvement of the effector arm of the immune system in antibody-mediated anti-tumor activity, primarily by engagement with activation and inhibitory Fc receptors, has been demonstrated (25). The extent to which cell-mediated or complement-dependent effector function mechanisms contribute to the anti-tumor activity exhibited by anti-PSCA mAbs needs to be examined. Similar anti-tumor activity was demonstrated by both 1G8 and 3C5 mAbs, the isotypes of which (γ1 and γ2a, respectively) differ in their ability to engage the immune system (26, 27). The 1G8 mAb was consistently more efficacious in growth retardation of established orthotopic LAPC-9 tumors, leading to greater prolongation of survival as compared with the 3C5 mAb. These results suggest an intrinsic antibody activity on tumor cells after binding to PSCA, which results in a significantly reduced and long-lasting tumor-growth rate. The increased efficacy exhibited by 1G8 as compared with 3C5 can be attributed to its higher affinity to PSCA and/or to its binding to a unique epitope on the middle domain of PSCA extracellular domain.

PSCA is one of the few largely prostate-specific cell-surface antigens that represent potential antibody therapy targets for prostate cancer. Other antigens include STEAP, an antigen highly expressed in all stages of prostate cancer including advanced hormone-refractory disease (14) and prostate-specific membrane antigen, which is expressed in early and late-stage prostate cancer (28). Of importance is the significant PSCA expression on the majority (80–100%) of patient specimens derived from all stages of the disease including androgen-refractory and metastatic tissues (9). These observations, together with the results presented in this study, validate PSCA as an attractive target for immunotherapy in prostate cancer and demonstrate the potential therapeutic utility of anti-PSCA mAbs for the treatment of recurrent and metastatic disease.

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