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Prostate cancer is the second leading cause of cancer-related death in American men. New treatments are needed. Prostate stem cell antigen (PSCA) is a cell surface protein overexpressed by a 40% of localized and as many as 80-100% of metastatic prostate cancers. A monoclonal antibody generated in our lab against PSCA inhibits growth and metastasis of prostate cancer in animal models, but the mechanism of action is not known. The goals of this grant are (1) to determine the mechanism of therapeutic activity monoclonal antibodies against PSCA and (2) to enhance the activity of these antibodies using combination approaches.

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INTRODUCTION

PSCA is a cell surface antigen expressed by a majority of prostate cancers. We have previously shown that PSCA is expressed at particularly high levels in prostate cancer bone metastases, which led us to hypothesize that it might be a suitable target for antibody therapy. We then went on to show that, indeed, antibodies against PSCA have significant anti-tumor activity, inhibiting prostate cancer growth and blocking prostate cancer metastasis in numerous in vivo models. The biological role of PSCA and the mechanism of therapeutic activity of PSCA antibodies are not known. Therefore, the goal of this proposal was to determine the mechanism of anti-tumor activity and try to use this information to improve the therapy. In particular, the goal of this grant was to determine if PSCA antibodies work via stimulation of the immune system or by direct binding of the antibody to PSCA on the cell surface. To date, we have made tremendous progress on our proposal.

PROGRESS REPORT

Specific Aim 1. To understand the therapeutic activity of monoclonal antibodies directed against PSCA in preclinical models.

Task 1. Whole antibody vs. F(ab')2. The goal of this task was to compare in vivo efficacy of whole PSCA antibody 1G8 to its F(ab')2 fragment, thereby testing whether the Fc portion of the antibody was required for antitumor activity. The first and difficult part of this task was to generate F(ab')2 fragment and demonstrate its purity and its continued ability to recognize PSCA. This was successfully done and we showed both that there was less than 0.5% contamination and that the F(ab')2 fragment still recognized PSCA on the cell surface by FACS. We then proceeded to test the fragment and whole antibody in the LNCaP-PSCA and the LAPC 9 models of prostate cancer. Two types of experiments were performed. In the first, antibody and tumor were given concurrently in order to test the ability of antibody to inhibit tumor formation. In the second, antibody was given to animals with established tumors in order to determine the effect of antibody on tumor growth. Molar equivalents of antibody were given. The results for LAPC 9, which expresses endogenous PSCA, are shown below in Figure 1. The results show that F(ab')2 fragments inhibit tumor growth as well as whole antibody, demonstrating convincingly that the Fc portion of the antibody is not required for tumor inhibition and that the antibody works by crosslinking of antigen, suggesting a direct mechanism of action.

Task 2: Comparison of antibody in FcR deficient mice.

A parallel way to approach the question of Fc dependence or independence is to administer whole antibody in mice lacking FcR. These mice were obtained from Jeff Ravetch at Rockefeller University. Although these experiments have been difficult secondary to the very slow breeding of these mice and the fact that we can only use male mice, we have managed to evaluate antibody 1G8 in a small cohort of FcR deficient mice

and found that the results were in agreement with those of Task 1, namely that the antibody was still able to inhibit tumor growth in the absence of FcR (data not shown). These experiments bolster the conclusion that PSCA antibody works through a direct mechanism of action.

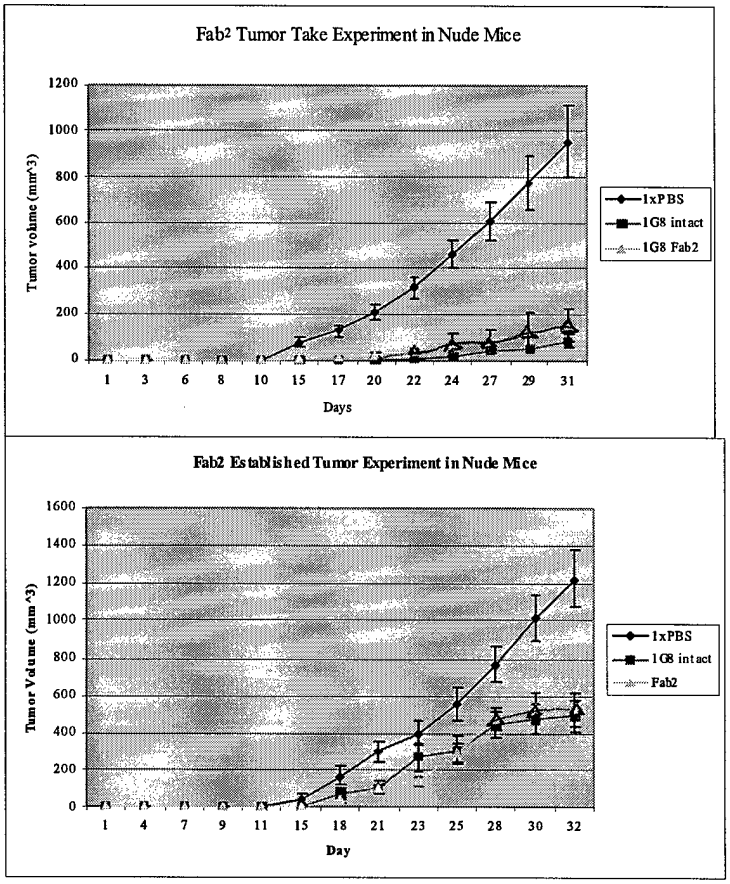

Figure 1. Comparison of whole antibody 1G8 and its F(ab')2 fragment in nude mice with LAPC 9 prostate tumors. In the top panel, antibody was given twice weekly beginning at the time of tumor inoculation. In the bottom panel, antbody was given when tumors reached ~ 0.5 cm 3 in size. As is seen, F(ab')2 fragment was equally effective as whole antibody in inhibiting tumor formation and growth.

Task 3: Additional in vivo assays. These experiments were designed as fallbacks if Tasks 1 and 2 did not work, and are not needed at this point. We have, however, begun to examine the mechanism of action of PSCA antibody 1G8 in in vitro model systems. We fund that the antibody can inhibit growth of LNCaP-PSCA cells in vitro, consistent with the direct mechanism of action we have proposed. Preliminary data suggest that antibody works by stimulating cells to undergo apoptosis (Figure 2). We also show that this is a nonclassical apoptotic pathway, independent of caspases. We also show that crosslinking of antigen is necessary, as single chain F(ab) antibody does not induce cell death (data not shown).

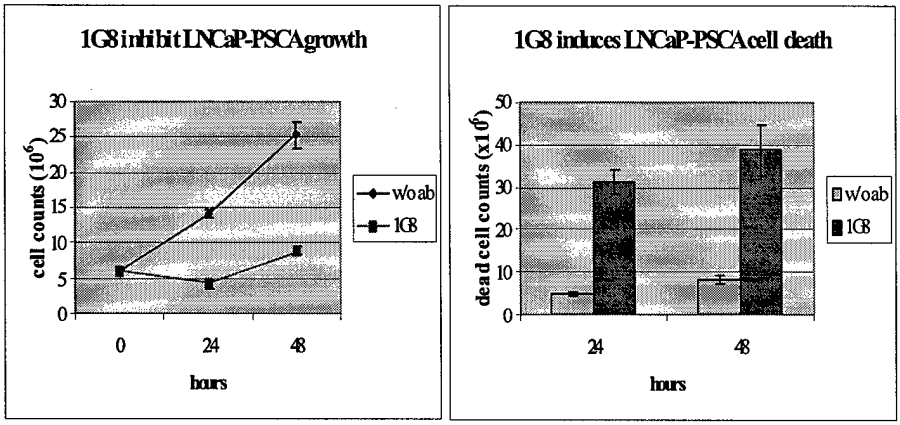

Figure 2. 1G8 can inhibit growth of LNCaP-PSCA cells in vitro (left panel) over 48 hours. On the right, we whow that this occurs because of increased cell death after antibody treatment, with 30-40% of cells dying at 24-48 hours. We show in other experiments that this cell death occurs as early as 6 hours after antibody exposure

Task 4 Comparison of antibody activity in tumors expressing high and low levels of PSCA. These experiments are in progress.

Specific Aim 2: To enhance the therapeutic acitivity of monoclonal antibodies directed against PSCA.

Task 1: Hormonal therapy + antibody. These experiments are in progress at this time. Preliminary results suggest that antibody can synergize with hormonal therapy to delay tumor growth.

Tasks 2-3. These experiments have not yet begun. There may be problems in obtaining GM-CSF for these studies secondary to cost. The supplier we initially planned on using no longer makes recombinant murine GM-CSF. Other vendors list prices that would cost as much as $1,000/mice. We are therefore exploring alternative drug combinations as well as looking at other ways to make or obtained GM-CSF.

KEY RESEARCH ACCOMPLISHMENTS OVER YEAR 1:

1. Completion of most of Aim 1, with demonstration of a direct mechanism of action of PSCA antibody therapy.

REPORTABLE OUTCOMES

The results discussed here are being put together for publication at this time. We expect to have a publication accepted by next year's report.

CONCLUSION: We have made significant progress over the past year. The major accomplishment has been the almost definitive demonstration that PSCA antibodies are acting independent of the immune system to inhibit tumor growth. These results suggest that PSCA itself may play an important role in tumor formation or growth, a hypothesis being explored using the antibody and also by other methods. Over the next two years, we expect to uncover more about the mechanism of action and also to explore ways to improve this therapy for translation into the clinic.