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13. ABSTRACT (Maximum 200 Words)

The goal of the proposed studies is to extend our OX40-specific anti-tumor responses to prostate tumor models by using a protein found on the surface of the T helper subset of leukocytes (OX40). Anti-OX40 delivered into animals with ongoing immune responses are able to clear the tumors and pathogens quicker following the acute immune response and also are left with a greater amount of immunologic "memory". The greater number of memory T cells patients have that recognize these tumors, increases their chance to fight off subsequent metastatic disease. We have found that prostate cancer patients treated with androgen ablation have a large influx of leukocytes into their prostate gland. These cells enter the prostate gland to recognize and destroy tumor cells. Leukocytes that invade the prostate gland after androgen ablation are $OX40^+$. Therefore, we hypothesize that androgen ablation followed by anti-OX40 treatment will enhance anti-tumor immunity in these patients and we propose to exploit our discovery in prostate cancer mouse model. Fine-tuning our approach to gain preclinical data will help us to understand the most efficient way to augment anti-prostate cancer immunity in prostate cancer patients for future clinical trials.

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INTRODUCTION: OX40 is a cell surface TNF-receptor family member that is primarily found on activated $CD4^+$ T cells. Our lab has shown that when this receptor is engaged through its ligand or an anti-OX40 Ab it induces proinflammatory signals that are clinically advantageous when delivered in combination with tumor vaccines or in mice with existing tumors. We also have found expression of OX40 on T cells infiltrating human primary tumors for head and neck cancer, breast cancer, melanoma, and colon cancer. Recently, in collaboration with Dr. Eugene Kwon we have found a large increase of $OX40^+$ T cells in prostate tumors of patients that had androgen ablation twenty days prior to surgery. We now feel there is compelling evidence that OX40 engagement in patients with prostate cancer could have beneficial effects leading to improved clinical results. Therefore, we hypothesize that OX40 engagement in vivo will enhance anti-prostate cancer immunity leading enhanced tumor-free survival. The objective of this study is to obtain enough preclinical data to warrant the design of an OX40-specific clinical trial in patients with prostate cancer. The specific aims of the study are as follows; 1) To determine whether OX40 engagement in vivo will enhance anti-prostate cancer specific immunity, 2) Can OX40 engagement in vivo enhance adoptive immunotherapy against prostate cancer, and 3) To investigate whether the combination of androgen ablation and anti-OX40 treatment are synergistic in the treatment of primary prostate cancer in TRAMP mice. The study design will determine whether OX40-specific tumor immunotherapy treatment regimens will be therapeutic in the TRAMP mouse tumor model. We will look at the efficacy of anti-OX40 therapy in both a tumor transplant setting and in transgenic TRAMP mice destined to succumb to prostate cancer through endogenous tumor formation. The molecular mechanism of anti-OX40 enhanced tumor immunity will also be assessed by gene array analysis of T cells stimulated through OX40. We feel that using anti-OX40 in a prostate cancer setting will ultimately benefit an ongoing anti-prostate cancer immune response leading to enhanced immunity against disease that recurs. This is a therapy that is relatively non-toxic and takes advantage of the body's own defense against malignant cells. The experiments proposed will provide the preclinical data necessary to fine-tune our observations so that we will have a favorable chance to succeed in a prostate cancer clinical in the future.

BODY:

Task #5: Gene chip analysis of T cells isolated from lung met model.

Our initial attempts to treat the TRAMP-C1 cell line with anti-OX40 as a solo reagent were not successful. At best, we observed a delay in tumor growth, but eventually all of the mice would succumb to the TRAMP-C1 tumor. Therefore, we searched for ways to enhance the anti-OX40 therapy, by first identifying mechanisms that drive the OX40-enhanced response. This was accomplished by performing gene array experiments comparing RNA from Ag-specific T cells that received anti-OX40 versus control Ab in two different CD4 T cell adoptive transfer models. In particular, we were looking for cytokine receptor genes that might be upregulated via OX40

engagement on the Ag-specific T cells that could be potentially used to enhance OX40 stimulation by combination (OX40/ cytokine) therapy. In both models anti-OX40 greatly upregulated the IL-2 receptor (CD25) and these results were confirmed at the protein level by FACS analysis (Prell 2003, Lathrop 2004). We also found that the signaling subunit of the IL-12R (IL-12β2 subunit) was upregulated in both CD4 T cell models and we recently confirmed the IL-12R result on the protein level by FACS analysis of Ag-specific T cells isolated from the draining lymph nodes (see Figure 1).

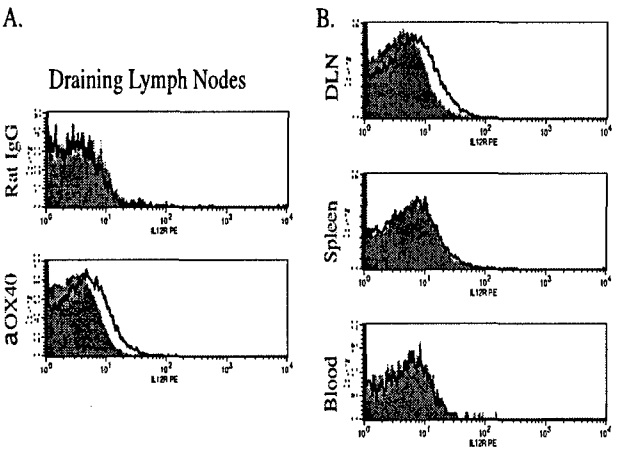

Figure 1. Anti-OX40 induced expression of IL-12Rb2 on antigen-specific CD4 T cells. Ovalbumin-specific DO11.10 CD4 T cells (3 X 10^6) were transferred (i.v.) into C57BL/6 recipients (day -1). One day later mice were injected with 500 ug soluble ovalbumin and 50 ug anti-OX40 or rat IgG s.c. (day 0). A second dose of 50 ug of anti-OX40 or rat IgG. Draining lymph nodes (DLN), spleens, and peripheral blood were taken on day 4 and the expression of IL-12Rb2 on the antigen-specific DO11.10 cells was measured by flow cytometry. **A.** DLN cells from anti-OX40 and rat IgG treated mice **B.** DLN cells, splenocytes, and peripheral blood from anti-OX40 treated mice.

Task #1: Timing dose titration experiments in the s.c. TRAMP tumor model.

Using the information obtained from the gene array experiments described above we performed anti-OX40/cytokine combination experiments to determine if there was any therapeutic synergy between IL-2 or IL-12 and anti-OX40 in the TRAMP-C1 prostate cancer model. We first tested the combination of anti-OX40 in combination with IL-2 and as shown in Fig. 2A there was no anti-tumor efficacy with either agent alone or when combined. However, when anti-OX40 was combined with IL-12 in tumor-bearing mice a significant delay in tumor growth was observed in 100% of the mice and 40% of the mice were tumor-free for 100 days after tumor inoculation (Fig 2B). Because anti-OX40 has strong proinflammatory effects on CD4 T cells, we determined whether CD4 T cell were essential for the anti-OX40/IL-12 treatment scheme. As shown in Fig 2B depletion of CD4 T cells prior to the anti-OX40/ IL-12 treatment scheme completely negated the therapeutic efficacy of the combined treatment. This

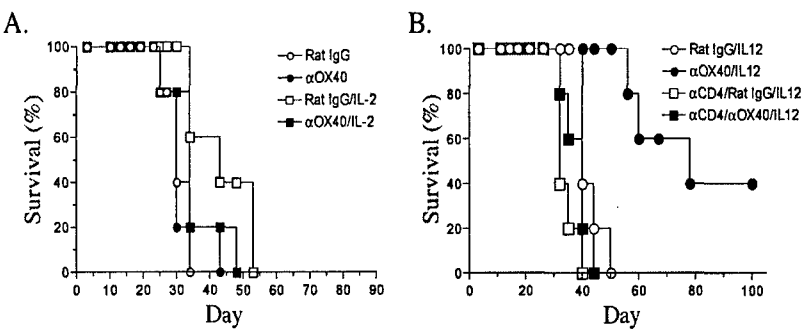

Figure 2. Anti-OX40 and IL-12, but not IL-2, synergize to improve survival of prostate tumor-bearing mice. **A.** Mice were injected s.c. in their right flanks with 7.5 X 10^5 TC1 tumor cells. Three days later mice were injected i.p. with 250 ug of anti-OX40 and 90,000 U rIL-2, or 250 ug rat IgG and 90,000 U rIL-2, or 250 ug anti-OX40 and PBS control, or 250 ug rat IgG and PBS control. Seven days after tumor challenge mice were given a second injection of 250 ug anti-OX40 or rat IgG. **B.** Half the mice were injected i.p. with 200 ug of anti-CD4 to deplete CD4 cells. One day later mice were injected s.c. in their right flanks with 7.5 X 10^5 TC1 tumor cells (day 0). On days 3 and 7 mice were injected i.p. with 250 ug anti-OX40 or rat IgG. Recombinant IL-12 (100 ng) was injected i.p. on days 4 through 9.

data suggest that anti-OX40 and IL-12 does enhance CD4 T cell immunity, which in turn has dramatic anti-tumor effects in vivo. These results are a significant step forward in our attempt to enhance anti-tumor immunity and currently we are testing this approach in TRAMP mice that spontaneously form tumors in vivo.

Task #6: Investigate androgen withdrawal in TRAMP mice.

Our initial observation in prostate cancer patients showed that androgen ablation prior to prostate cancer surgery greatly increased the numbers of $OX40^+$ T cells within the tumor of these patients. Therefore we wanted to test whether the combination of androgen withdrawal and anti-OX40 would show enhanced anti-tumor effects in mice with TRAMP tumors. As shown in Fig 3, we have tested anti-OX40 in combination with androgen ablation (castration) versus androgen ablation alone in the subcutaneous TC1 model and saw no increase in therapy with the combined treatment. We will also attempt this strategy in the TRAMP mice that spontaneously develop tumors and will most likely include IL-12 treatment with the androgen ablation/anti-OX40 treatment.

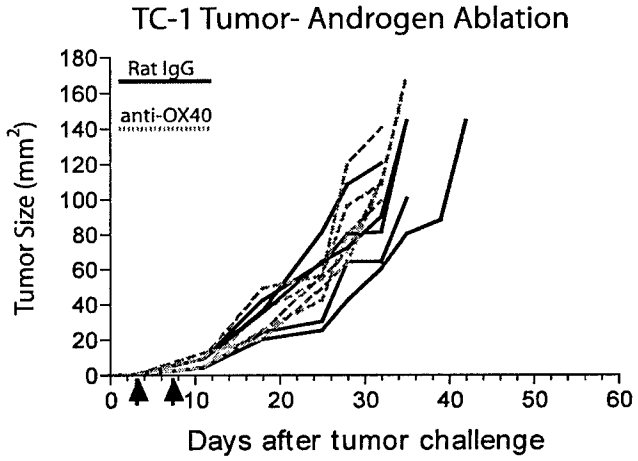

Fig 3. *Anti-OX40 treatment had no effect of TC-1 tumor growth in androgen ablated animals.* Six-Eight week old male C57BL/6 male mice were castrated and allowed to recover for seven days. Mice were then challenged with 7.5 X 10^5 TC-1 tumors injected s.c. on the right flank. Anti-OX40 (250 µg) or rat IgG control were injected i.p. three and seven days following tumor challenge (arrows), and monitored for tumor growth. Mice were sacrificed if tumors were ulcerated or if tumor growth reached 150mm^2. (n=5 per group)

KEY RESEARCH ACCOMPLISHMENTS:

1) Discovering that both the IL-2 receptor and IL-12 receptor are upregulated following anti-OX40 engagement in vivo.
2) Using the information above to try anti-OX40/cytokine combinations in the TRAMP-C1 prostate tumor model, which was not responsive to anti-OX40 alone.
3) The combination of anti-OX40 and IL-12 proved to be a potent therapy in mice harboring the TRAMP-C1 tumor.
4) The anti-OX40/IL-12 was dependent on CD4 T cell function for its therapeutic function against the TRAMP-C1 tumor.
5) Initial attempts to combine androgen ablation with anti-OX40 therapy in the TRAMP-C1 tumor showed no additive or synergistic anti-tumor activity.

REPORTABLE OUTCOMES:

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Immunology. 112:559.

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CONCLUSIONS:

During the second year of funding for the DOD prostate cancer work we have targeted an OX40-based prostate cancer therapy that involves the addition of IL-12. We had previously found that the addition of anti-OX40 alone only slightly delayed the growth of the TRAMP-C1 prostate tumor line in mice. Through a series of gene array studies we found that anti-OX40 specifically upregulated two cytokine receptors on Ag-specific CD4 T cells: IL-2 receptor (CD25) and IL-12R β2 subunit. Therefore we used this knowledge to attempt combination therapies with anti-OX40 and IL-2 or IL-12. As shown in Figure 2 the combination of anti-OX40 and IL-2 had no effect on TRAMP-C1 tumor growth, however the combination of anti-OX40 and IL-12 had dramatic anti-tumor activity leading to 40% tumor-free mice. We have now taken this observation and tested anti-OX40/IL-12 therapy in TRAMP mice that spontaneously form prostate tumors 20-30 weeks after birth. Within the coming month we will examine the TRAMP mice receiving the combination therapy. We hope to publish these novel findings within the coming year. One of the original ideas of the initial application was to ascertain whether the combination of androgen ablation and anti-OX40 would be additive or synergistic in tumor-bearing mice. We tested this theory in mice bearing the TRAMP-C1 tumor and found that androgen ablation alone or in combination with anti-OX40 did not increase anti-tumor activity.

Ultimately, this work will complement the anti-OX40 phase I clinical trial that should be initiated soon, pending final FDA approval, and knowledge we are learning from this preclinical research could be translated into a phase II/III clinical trial for prostate cancer patients in the future.