Targeting lymphotoxin-mediated negative selection to prevent prostate cancer in mice with genetic predisposition

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The identification of individuals genetically susceptible to cancer calls for preventive measures to minimize the cancer risk in these high-risk populations. Immune prevention is made necessary by the anticipated health threat, but lack of enough high-affinity T cells against tumor-associated antigens and the unpredictability of tumor antigens make antigen-based immune prevention untenable for cancer. To address this issue, we explored a non-antigen-based cancer immune prevention strategy using the transgenic adenocarcinoma of mouse prostate model that spontaneously develops prostate cancer with 100% penetrance. We show that targeted mutation of the lymphotoxin α ($LT\alpha$) gene efficiently rescued tumor-reactive T cells, drastically reduced cancer incidence, and almost completely ablated metastasis. Remarkably, short-term treatments with the fusion protein consisting of constant region of IgG and extracellular domain of lymphotoxin β receptor (LTβRIg) interrupted clonal deletion, reduced the size of the primary cancer, and completely prevented metastasis later in life. Our data demonstrated the value of non-antigen-based immune prevention for those with a genetic predisposition to cancer.

prostate cancer mouse model | T-cell development | T-cell effector function | non-antigen-based immune prevention

One of the most important advances in cancer research is the identification of individuals with increased susceptibility to cancer development (1). Broadly speaking, genetic susceptibility can be conferred by inactive alleles of tumor suppressor genes or by hypermorphic alleles of oncogenes (2, 3). In extreme cases, inactivating mutations of tumor suppressor genes such as *p53* (4), the adenomatous polyposis coli (*APC*) gene (5, 6), and *BRCA1/2* (7–9) can result in a nearly 80% lifelong cancer risk. Traditionally, high-penetrance risk alleles allowed identification of cancer-associated genes. Family history alone, however, can serve as a powerful tool to identify an individual with high risk. A comprehensive study involving >2 million nuclear families revealed that individuals with an affected sibling and at least one affected parent can have a >30-fold higher risk of developing colon-rectal cancer (10). Although it is estimated that ≈1–5% of cancer cases are caused by dominant familial susceptibility alleles, the majority of the genes associated with cancer risk remain to be identified (11). Recent genome-wide association studies, however, have allowed the identification of numerous susceptibility loci (12–17). It is anticipated that increasing numbers of individuals will be diagnosed with high cancer risk, providing an enormous need for the identification of cancer preventative therapies.

Identification of genetically susceptible individuals thus calls for preventive measures to minimize cancer risk in these high-risk populations (18). Although prophylactic surgery and chemoprevention exist as viable options for the prevention of cancer, prophylactic surgery is difficult to implement to those yet to develop cancer given the vital importance of many organs, and chemoprevention has a high burden of compliance and drug safety. There-fore, other preventive options are highly warranted. Thanks to immunological memory, immunity can last a lifetime without the stringent requirement of frequent boosting. Unfortunately, we are not aware of any attempt to use immune prevention to reduce both risk and mortality of cancer among the population of individuals with a high genetic predisposition to cancer.

Immune prevention is made necessary by the anticipated health threat and possible by predictability of antigens carried by pathogens. The classic notion of immune prevention is based on immunization with antigens expressed by the pathogens. The power of immune prevention is best demonstrated by the large-scale prevention of various infectious diseases, including eradication of smallpox. However, adoption of immune prevention to cancer is limited by several factors. First, compared with pathogens, cancer antigens are poorly defined, unpredictable, and more heterogeneous (19–21), which makes it considerably more difficult to design antigen-based vaccines for the purpose of prevention. Second, because cancers are derived from normal tissues, most of the high-affinity T cells reactive to such peripheral tissue antigens in the cancer cells have been deleted (22). Lack of high-affinity tumor-reactive T cells would in theory make immune prevention difficult to attain.

Recent studies have demonstrated that clonal deletion of T cells reactive to peripheral antigens depends on their expression in thymic medullar epithelial cells (23, 24). Because tumors are composed of malignantly transformed cells from normal tissues, and therefore likely express tissue-specific antigens, it is of interest to determine whether these T cells can be rescued for the purpose of immune prevention. Because the lymphotoxin α ($LT\alpha$) gene plays a major role in the development and function of medullar epithelial cells (25, 26), especially in the context of clonal deletion of peripheral antigen-reactive T cells, blocking this pathway may allow one to rescue tumor-reactive T cells to prevent the development of cancer. By using mice with a targeted mutation of $LT\alpha$ (27), we reveal here a valuable target for rescuing prostate cancer-reactive T cells and a means of cancer immune prevention. More importantly, transient blockade of $LT\alpha$ significantly reduced the size of the prostate cancer tumor and eliminated cancer metastasis. To our knowledge, this strategy represents the first non-antigen-based means of immune prevention for cancer and has a realistic chance to be translated into clinical care of those patients with high genetic risk for cancer.

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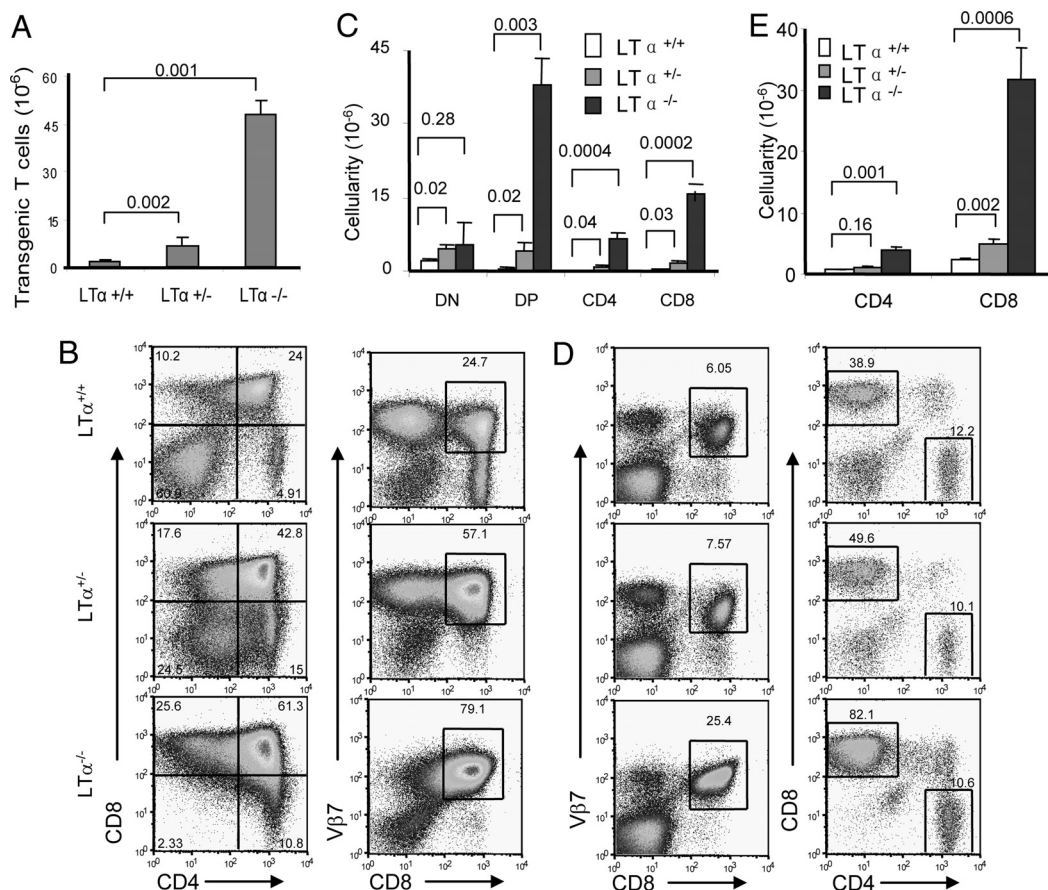
Fig. 1. LTα deficiency prevents clonal deletion of tumor-reactive T cells in TRAMP mice. LTα$^{+/+}$, LTα$^{+/-}$, and LTα$^{-/-}$ Tag-ITRAMP mice were killed at 6 weeks for analyses. Thymocytes (A–C) and splenocytes (D and E) were harvested and analyzed by flow cytometry, using antibodies specific for Vβ7 (transgenic TCRβ), CD4, and CD8. (A) Number of Vβ7$^+$ cells in the thymus. Data shown are means and SEM of cell numbers (n = 8). (B) Plots depicting the distribution of CD4 and CD8 markers among the Vβ7$^+$ thymocytes (*Left*) or CD8 and Vβ7 among total thymocytes (*Right*). (C and E) Number of different subsets of transgenic Vβ7$^+$ T cells in the thymi (C) and spleens (E). Data shown are means and SEM of cell numbers (n = 8). (D) Plots depicting the distribution of CD8 and Vβ7 markers among total splenocytes (*Left*) or CD4 and CD8 markers among the Vβ7$^+$ splenocytes (*Right*). The data in the left images are from one representative mouse per group, and similar data were obtained in two independent experiments, involving a total of eight mice per group.

Results

**Targeted Mutation of *LTα* Limits Clonal Deletion of SV40 T Antigen-Specific T Cells.** One of our groups has recently demonstrated a critical role for LTα in the clonal deletion of T cells specific for tissue-specific antigens (25). As a first test to determine whether this pathway can be exploited for cancer immune prevention, we took a transgenic approach to determine whether this pathway can rescue cancer-reactive T cells. We crossed transgenic mice expressing a T-cell receptor (TCR) specific for SV40 large T antigen (Tag-I) (28) to transgenic adenocarcinoma of mouse prostate (TRAMP) mice expressing SV40 large T antigen under the control of a probasin promoter (29), with the null mutation in none of the alleles, one allele, or two alleles of the *LTα* gene. The development of the transgenic T cells was evaluated by flow cytometry.

As shown in Fig. 1*A*, in the Tag-I/TRAMP double-transgenic mice, targeted mutation of one or both alleles of the *LTα* gene resulted in a significant increase in total thymic cellularity. A dramatic increase in the percentage of CD4$^+$CD8$^+$ (DP) cells and a significant decrease in the percentage of CD4$^-$CD8$^-$ (DN) cells was observed among the transgenic TCR$^+$ cells. Targeted mutation of both alleles of *LTα* eliminated the DN subset, whereas the DP and CD8 single-positive (SP) subsets expanded (Fig. 1 *B* and *C*). In addition, the numbers of transgenic T cells were greatly increased in the spleens of the *LTα*-deficient mice (Fig. 1 *D* and *E*). Remarkably, partial rescue was observed in the heterozygous mice

(Fig. 1). Therefore, *LTα* plays a critical role in the clonal deletion of SV40-large T antigen-reactive T cells.

Targeted Mutation of *LTα* Inhibits the Development of Spontaneous Prostate Cancer. To test a role for LTα in the onset of prostate cancer, the size of the prostates of the *LTα*-deficient TRAMP mice were measured at 30 weeks of age by MRI (30). Representative images are shown in Fig. 2*A*, whereas the summary data are shown in Fig. 2*B*. These data demonstrated that the size of the prostate was reduced by more than threefold in the TRAMP mice with either heterozygous or homologous deletion of *LTα* (Fig. 2*B*). At 34 weeks, the three groups of mice were killed for double-blind histology analyses of cancer development and metastasis. As shown in Fig. 2*C*, 100% of the WT mice developed malignant prostate cancer, with metastasis in 7 of 12 cases. Among them, one mouse had metastasis in the kidney only, whereas six others had metastasis in the lung, including two that also had metastasis in the liver. In mice harboring the homozygous mutation, only 45% (5 of 11) mice developed malignant tumors. Remarkably, 4 of 11 mice had normal prostate morphology, whereas 2 others had prostate intraepithelial neoplasia (PIN). Only 1 of 11 mice had metastasis, in both the liver and lung. A reduction of cancer incidence (13 of 16) was also observed in the heterozygous mice. Two heterozygous mice had completely normal prostates and one mouse had PIN. Moreover, only 1 in 16 heterozygous mice showed lung metastasis. Because
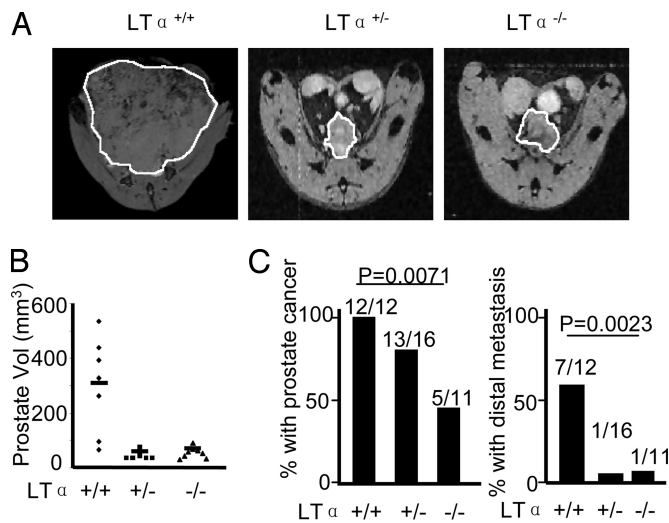
Fig. 2. LTα deficiency inhibits development of prostate cancer. The tumor incidence of Ltα$^{+/+}$TRAMP, Ltα$^{+/-}$TRAMP, and Ltα$^{-/-}$TRAMP mice were diagnosed by double-blind histology examination by two individuals at 34 weeks, whereas the prostate volumes were measured by MRI at 30 weeks. (A) Representative local prostate images of Ltα$^{+/+}$TRAMP, Ltα$^{+/-}$TRAMP, and Ltα$^{-/-}$TRAMP mice. The prostate is identified with thick white outlines. (B) The prostate sizes of Ltα$^{+/+}$TRAMP, Ltα$^{+/-}$TRAMP, and Ltα$^{-/-}$TRAMP mice at 30 weeks of age. (C) Targeted mutation of LTα resulted in reduction of prostate cancer incidence and elimination of distal metastasis. The raw data for incidence are provided on the top of bars, whereas the P values shown in the images are obtained by χ^2 analyses for gene dose effects. The malignancy and metastasis were diagnosed by two independent and double-blind evaluations of at least three slides per organ, including, heart, liver, lung, kidney, pancreas, and intestine, 25 μm apart.

development of lymphatics is normal in the LTα-deficient mice (27), lack of metastasis cannot be attributed to abnormality in the lymphatics. χ^2 analysis indicates a gene dose-dependent reduction both in the rate of malignancy ($P = 0.0071$) and metastasis ($P = 0.0023$). Taken together, our data presented in Figs. 1 and 2 demonstrate that targeted mutations of $LT\alpha$ rescue tumor-reactive T cells and increase host resistance to prostate cancer.

The Administration of LTβRIg Rescues Tumor-Reactive T Cells Without Provoking Autoimmune Inflammation. The fact that genetic inactivation of LTα conveys host resistance to prostate cancer raised an interesting possibility that $LT\alpha$ may be targeted for the purpose of immune prevention. Because aged $LT\alpha^{-/-}$ mice developed chronic inflammation, one has to be concerned with potential autoimmune side effects of this treatment (25, 26). To achieve this goal, we compared the inflammatory response when mice were treated with three weekly doses of soluble murine LTβRIg or human IgGFc, starting at 4, 6, or 11 weeks of age. The mice were killed 4 weeks after completion of the treatments. As shown in Table 1 and Fig. 3, whereas infiltrates in liver and lung were observed in mice that received their first dose at 4 weeks, no inflammation or tissue injury were observed when the treatment was initiated at 6 or 11 weeks.

We have recently reported the existence of strong clonal deletion in double-transgenic TRAMP/TGB mice that express TCR specific for SV40 large T antigen (31, 32). The clonal deletion was characterized by a massive reduction of CD8$^+$Vβ8^{hi} transgenic T cells (31). These features were recapitulated in the double-transgenic mice receiving IgG Fc control (Fig. 4A). Interestingly, treatment with LTβRIg resulted in a 6-fold increase in the DP and a nearly 3-fold increase in the CD8 SP subset (Fig. 4 B Lower and C). Correspondingly, the number of transgenic CD8 T cells was more than doubled in the spleen (Fig. 4 D and E). The increase in CD4 T cells in the thymus and spleen is consistent with the notion that

Table 1. Inflammation induced by LTβRIg at 4 weeks, but not 6 or 11 weeks

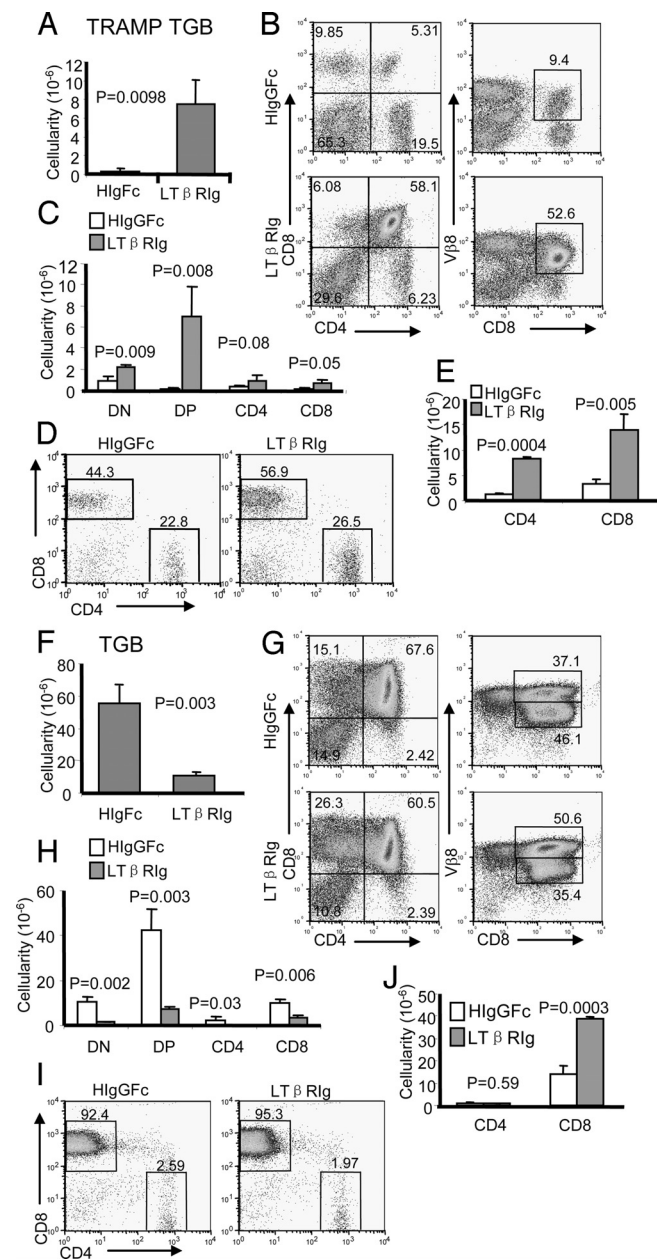|	Organ/treatment							
	Liver		Lung		Kidney		Prostate	
	hIg	LTβRIg	hIg	LTβRIg	hIg	LTβRIg	hIg	LTβRIg
4 weeks	0/7	6/7	0/7	3/7	0/7	0/7	0/7	0/7
6 weeks	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
11 weeks	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3

h, human.

rescue of clonal deletion occurred at DP. The increase of transgenic TCR$^+$ DN (which differed from normal DN) can also be attributed to less severe clonal deletion in the LTβRIg-treated mice. In mice lacking the large T antigen, no increase of transgenic T cells in the thymus was conferred by the fusion protein (Fig. 4 F–H). In contrast the fusion protein actually reduced the number of transgenic T cells in the thymus. Therefore, the LTβRIg expanded SV40 T antigen-specific T cells only if the antigen was present.

To determine whether LTβRIg prevented deletion of antigen-specific T cells, we compared the percentage of apoptotic cells by staining with annexin V. As shown in Fig. 5, LTβRIg significantly reduced the percentage of apoptotic cells regardless of the subsets of the transgenic thymocytes. This treatment, however, had no effect on apoptosis of T cells in the spleen. Therefore, the increase of transgenic T cells in the TRAMP/TGB mice is likely due to rescue of T cells from clonal deletion in the thymus.

Short-Term Treatment with LTβRIg Reduces the Progression of Primary Prostate Cancer and Prevents Metastasis. LTβRIg binds LTα with high affinity. To test whether LTβRIg treatment can significantly affect the progression of prostate cancer, we treated the TRAMP mice with three weekly injections of either LTβRIg or
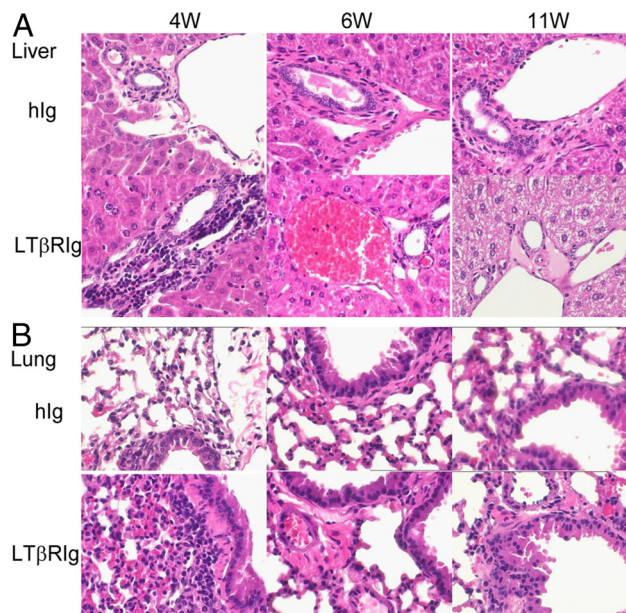
Fig. 3. Identification of a time window to avoid lymphocyte infiltration associated with LTβRIg treatment. Four-, 6-, and 11-week-old C57BL/6 mice received three weekly i.p. injections with 100 μg of either soluble murine LTβRIg or human IgGFc. The mice were killed 4 weeks after the last injection. Peripheral organs were collected for H&E staining. (A) Lymphocyte infiltration into the liver was only observed when the treatment was initiated at 4 weeks, but not at 6 or 11 weeks. (B) Infiltration to the lung was only observed if the treatment was initiated at 4 weeks of age.

Fig. 4. LTβRIg treatment rescued tumor-reactive T cells from clonal deletion in the thymus. TRAMP/TGB (*A–E*) or TGB (*E–J*) transgenic mice received three weekly injections (i.p.) of 100 μg of either soluble LTβRIg or human IgGFc, starting at 6 weeks of age. The mice were killed 2 weeks after the last injection. Thymocytes (*A–C* and *F–H*) and splenocytes (*D, E, I,* and *J*) were harvested and analyzed by flow cytometry using antibodies specific for CD4, CD8, and Vβ8. (*A* and *F*) Number of Vβ8+ thymocytes. (*B, D, G,* and *I*) Representative plots depicting distribution of CD4, CD8, and transgenic TCRβ. Plots are from gated Vβ8+ cells, except for the two right images in *B*, which represent that of total thymocytes. Similar data were obtained from two independent experiments, each involving four mice per group. The numbers of different subsets of Vβ8+ transgenic thymocytes (*C* and *H*) and splenocytes (*E* and *J*) are presented in bar graphs as means and SEM, involving eight mice per group.


Fig. 5. LTβRIg reduced apoptosis of transgenic T cells in the TRAMP/TGB transgenic mice. Thymocytes and splenocytes of the TRAMP/TGB mice as described in Fig. 4 were stained with antibodies against Vβ8, CD4, and CD8 in conjunction with annexin V. (*A*) LTβRIg treatment on TRAMP/TGB mice reduced the percentage of apoptotic cells in the thymus, mainly at the DP stage. (*B*) LTβRIg had no impact on apoptosis of transgenic T cells in the spleen. The plots depict apoptotic cells among different subsets of thymocytes (*A*) and spleen cells (*B*). The numbers in the images are means and SEM of the percentage of apoptotic cells, summarized from two independent experiments, each with four mice per group (*n* = 8).

control IgGFc, starting at 6 weeks. At 30 weeks, the volume of the prostate was measured by MRI. As shown in Fig. 6*A*, on average, the LTβRIg treatment at 6 weeks caused a >50% reduction in the prostate volume (*P* < 0.01).

We carried out histological analysis to characterize the effect of LTβRIg treatment on the development of metastasis. As shown in Fig. 6*B*, four of seven control-Ig-treated TRAMP mice developed metastasis in the lung and/or liver, consistent with previous reports by others (29). Importantly, none of the LTβRIg-treated mice developed metastasis. Moreover, the lack of autoimmune disease is further supported by lack of inflammation in any of the organs studied (Fig. 6*B*) (data not shown). Therefore, transient treatment of LTβRIg reduced the size of the primary lesion and completely prevented metastasis without provoking lymphocyte infiltration into organs.

Discussion

It is difficult to use cancer vaccines as preventive measures for those individuals with a genetic predisposition to cancer because of the multitude of mechanisms of immune tolerance, including clonal deletion to tissue-specific antigens (22, 31, 32) and clonal anergy (33) as well as the unpredictability of tumor antigens (19–21). Here, we devised a non-antigen-based strategy of immune prevention that in theory can be applicable to tumors from a variety of tissue origins. The foundation of the strategy is the critical role of LTα in the clonal deletion of T cells specific for peripheral antigens (25, 26). By using TCR transgenic mice as the basic readout, we have demonstrated that short-term treatments with soluble LTβRIg rescued cancer-reactive T cells
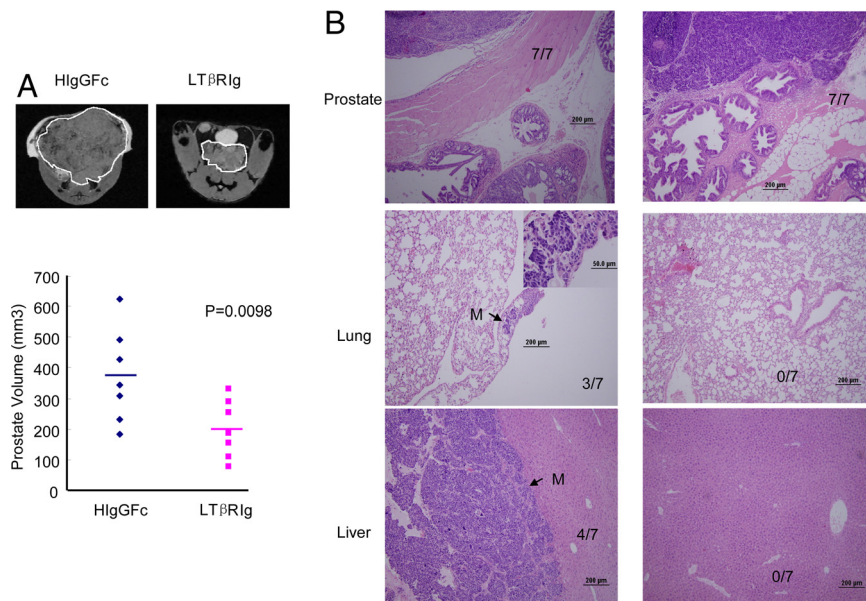
Fig. 6. LTβRIg treatment reduced size of prostate cancer and prevented metastasis. (*A*) Prostate volumes as measured by MRI. Male TRAMP mice received three weekly i.p. injections with either 100 μg of soluble murine LTβRIg or human IgGFc at 6 weeks of age. The prostate volume was measured at 30 weeks. The upper images show representative local images of human IgGFc-treated and LTβRIg-treated TRAMP mice. The prostates are identified with thick white outlines. The lower images depict the sizes of individual prostates (*n* = 7). (*B*) Histological analysis of tumor metastasis. TRAMP mice that received three weekly treatments of control Ig or LTβRIg starting at 6 weeks were killed at 33 weeks after MRI analysis at week 30. H&E sections were examined double blind by a pathologist for metastatic lesions in all internal organs, including liver, lung, kidney, colon, heart, and pancreas. Metastases (to lung and/or liver) were found in four of seven control Ig-treated and none of the LTβRIg-treated mice. The differences in the rate of metastasis are statistically significant (*P* = 0.012).

that would be otherwise deleted in the thymus. Corresponding to this, we found that TRAMP mice that received short-term treatment of soluble LTβRIg at 6 weeks had significantly reduced tumor sizes at 30 weeks. More importantly, this treatment completely prevented the development of metastasis. Because the antigens involved in tumor rejection are unknown, we have not been able to measure antigen-specific T-cell response in either tumor-infiltrating cells or spleen. Because the targeted mutation of *LTα* limits clonal deletion of SV40 T antigen-specific T cells and inhibits development of spontaneous prostate cancer, prevention by LTβRIg is likely due to its binding to LTα, although involvement of other potential LTβR ligand cannot be ruled out.

It has been demonstrated that transgenic mice expressing SV40 T antigens developed tumors concomitant with the development of T antigen-specific T cells (34). Therefore, merely priming antigen-specific T cells is insufficient to prevent tumor development. The quality of T cells, such as the antigens recognized and the affinity for cancer antigens, also likely matters. Our data presented in this study indicated that blockade of LTα can efficiently prevent deletion of two lines of high-affinity transgenic T cells specific for an antigen expressed in a prostate-specific fashion as a transgene.

A major advantage of the LTα blockade-based immune prevention is the potential applicability to a number of different cancer types regardless of tumor antigens involved. Although it remains to be tested whether this strategy is applicable to humans, it is of interest to note the association between LTα polymorphisms and risk of prostate cancer in humans (35–37).

It is of interest that, despite successful induction of cancer-reactive T cells based on unmutated tumor antigens, therapeutic tumor vaccines targeting self antigens have had limited impact (38). Several factors made it plausible that a general strategy to rescue tissue-specific T cells can be more effective, as demonstrated here. First, our basic strategy relies on rescuing high-affinity self-reactive T cells that are destined to be deleted in the thymus, whereas the traditional vaccination aims at expanding those T cells that have escaped clonal deletion, presumably because of their lower affinity. Second, the repertoire of T cells induced by vaccination in normal mice is likely more limited than what can be anticipated from those that have been treated to rescue cancer-reactive T cells from clonal deletion. Third, it is well known that cancer-bearing host and cancer environment do not favor a strong antitumor immune response.

One can reasonably surmise that the best time to induce cancer immunity is before the development of cancer.

An important consideration is the duration of the rescued cancer-reactive T cells before the development cancer. This will determine the time of initiation and the duration of the preventive regimens such as the one described herein. Because memory T cell responses can last for a lifetime, the key is to define a time window in which cancer-reactive T cells can be effectively rescued without triggering a significant autoimmune-associated side effect. Additional studies are needed to define such a window. Because a dramatic reduction of human thymic mass occurs during puberty (39), it may be preferable to rescue tumor-reactive T cells at an early age. However, thymopoiesis can be enhanced after chemotherapy or hormone ablation therapy (40); the treatment of host bearing dormant tumor can be started after intensive chemotherapy. It might be of interest to determine whether such a rescue of cancer-reactive T cells can control the relapse of tumor in adult life.

Because the prevention is to be applied to high-risk healthy patients, a primary concern is its potential for generating autoimmune side effects. It has been reported that a germ-line mutation of LTα causes multiple organ infiltration (25). Nevertheless, it is worth pointing out that no significant tissue destruction is observed in organs after careful histological examination. Likewise, the elevations of autoantibodies were modest in the LTα-deficient mice. More importantly, through 15 years of observations by one of our groups, no significant difference in either life span or body weight was observed in LTα-deficient mice (data not shown), as was originally described in ref. 27. Serum and urine chemistry measurement also failed to reveal destruction of liver or kidney (data not shown). These data demonstrated that autoimmunity, although demonstrable in the LTα-deficient mice (25), is not accompanied by severe autoimmune disease. Our analysis of the LTβRIg-treated mice indicated significant lymphocyte infiltration when the treatment was initiated at 4 weeks. However, no lymphocyte infiltration was observed if the treatment was initiated at 6 weeks or later. The side effects when treated at 4 weeks of age were probably due to the more active thymopoiesis that occurs at a younger age. Because treatment at 6 weeks had a significant preventive effect, our data demonstrate that it is possible to identify an appropriate window in which cancer immune prevention can be achieved without overt risk of autoimmune diseases. In this context, it is of interest to note a previous observation by Hara et al. (41), who showed that even

when an autoantigen was used to vaccinate, a dose can be found that triggers tumor rejection without autoimmune diseases.

Taking these findings together, this study has opened an avenue to develop an immune intervention that prevents cancer development. This approach represents a major departure from the principle of cancer vaccine because it alleviates the need to identify tumor antigens. It is envisaged that subjects that carry a high-risk allele may be treated with reagents to block $LT\alpha$ or other critical pathways for tolerance to peripheral antigens to reduce their future cancer risk and improve clinical outcome if they do develop cancer.

Materials and Methods

Experimental Animals. WT TRAMP mice expressing the SV40 T antigen (Tag) controlled by rat probasin regulatory elements and $Lt\alpha^{-/-}$ mice, all in the C57BL/6 background, were purchased from The Jackson Laboratory. The mice were bred at the animal facilities of the Ohio State University (Columbus, OH) and the University of Michigan (Ann Arbor, MI). Transgenic Tag-I and TGB mice expressing TCR specific for different epitopes of SV40 large T antigen presented by different MHC loci have been described in refs. 28 and 42.

The generation of TRAMP mice expressing TGB TCR (TGB-TRAMP) was described in ref. 31. $Lt\alpha^{+/+}$TRAMP, $Lt\alpha^{+/-}$TRAMP, and $Lt\alpha^{-/-}$TRAMP mice were obtained by breeding $Lt\alpha^{+/-}$ mice with $Lt\alpha^{+/-}$TRAMP mice. The Tag-I mice were bred with $Lt\alpha^{-/-}$ mice to obtain $Lt\alpha^{+/-}$Tag-I mice, which were crossed with the $Lt\alpha^{+/-}$TRAMP mice to produce $Lt\alpha^{+/+}$Tag-ITRAMP, $Lt\alpha^{+/-}$Tag-ITRAMP, and $Lt\alpha^{-/-}$Tag-ITRAMP mice.

LTβRIgFc Treatment. For cancer prevention, 6-week-old TRAMP mice were treated with three weekly injections of 100 μg of LTβRIgFc or control IgGFc (i.p.). Treated mice were examined at least weekly for a palpable tumor in the lower abdomen. The prostate volume was measured by MRI at 30 weeks. Mice were killed at 33 weeks, and internal organs were collected for histology analysis.

For rescue of clonal deletion, 6-week-old TRAMP/TGB mice were treated with three weekly injections of 100 μg of LTβRIgFc or control IgGFc (i.p.). Two weeks after the last treatment, the mice were killed and the total thymocytes and splenocytes were harvested and stained with fluorochrome-conjugated anti-CD4 (RM4.5), anti-CD8 (53-6.7), and anti-Vβ8.1+8.2 (MR5-2) antibodies and analyzed by flow cytometry (LSR; Becton Dickinson).

To test potential autoimmune side effects, 4-, 6-, and 11-week-old TRAMP mice were treated with 100 μg of LTβRIgFc or control IgGFc every week, for a total of three injections i.p. Two weeks after the last treatment, the mice were killed and peripheral organs were collected. Tissue sections from peripheral organs were stained with H&E.

MRI of Prostate. The progression of prostate cancer in the TRAMP model was measured by MRI as described in ref. 30.

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