Murine Prostate Cancer Inhibits Both In Vivo and In Vitro Generation of Dendritic Cells From Bone Marrow Precursors

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BACKGROUND. There is increasing evidence to suggest that dendritic cells (DC) are functionally impaired in tumor bearing hosts. However there is little or no data on the effects of murine prostate cancer (CaP) on DC generation from bone marrow precursors.

METHODS. Flow cytometry, mixed leukocyte reactions (MLR), and immunohistochemical analyses were used to characterize DC in CaP.

RESULTS. DC generated in the presence of CaP cell lines RM1 and the cell line C2 from the transgenic adenocarcinoma of the mouse prostate (TRAMP) mouse in a Transwell system expressed significantly lower levels of DC differentiation markers. This effect was confirmed when TK-neo-transfected RM1 cells were directly added to DC cultures and eliminated 5 days later using gancyclovir (GCV). Furthermore, co-incubation of DC with CaP cells resulted in a decrease in the stimulatory capacity of DC to induce T cell proliferation in the MLR assay. These results were further confirmed in vivo in two different murine models of CaP: i) DC generated from mice intrafemorally injected with TK-neo-transfected RM1 cells; and ii) in DC generated from TRAMP mice.

CONCLUSIONS. The generation and function of DC are significantly suppressed in the CaP microenvironment in both in vivo and in vitro murine models. Prostate 59: 203–213, 2004.

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INTRODUCTION

Prostate cancer (CaP) is the second leading cause of cancer death among American men. Although the majority of CaP cases are localized to the prostate, nearly a third of newly diagnosed patients have locally advanced or metastatic CaP [1]. The development and progression of tumors, including CaP, may in part be secondary to escape from immune recognition, and defective antitumor immunity [2,3]. Initiation of specific antitumor immunity requires involvement of professional antigen-presenting cells. Dendritic cells (DC) are the most potent antigen-presenting cells and have been shown to be capable of recognizing, processing,
and presenting specific antitumor immune responses, and initiating specific antitumor immune responses in both animals and humans [4]. Thus, adequate DC function is essential for the development of antitumor immunity. However, in tumor-bearing hosts the immune system frequently does not react to tumor-associated antigens and tumors, instead of being eliminated, progress, metastasize, and ultimately kill the host. One explanation for this sequence of events may be either local or systemic suppression of the DC system by tumor-derived factors, resulting in inhibition of immune responsiveness, or development of immune tolerance [2,5].

Patients with advanced CaP have been shown to have dysfunctional cell-mediated immunity characterized by a predominantly Th2 cytokine profile, with a lower proportion of cells producing IFN-γ and IL-2 and higher proportion of cells secreting IL-4 [6], impaired expression and function of signal-transducing zeta chain in peripheral T cells and NK cells [7], and other causes of impaired tumor detection by the immune system in CaP [8]. Clinical observations suggest that the number of DC in prostate carcinomas inversely correlates with the histopathological grade of the tumor [9]. Furthermore, the ability of DC from cancer patients to stimulate T lymphocytes was profoundly suppressed, explaining the failure of tumor-infiltrating lymphocytes to eliminate tumor cells [10]. It has recently been shown, that following co-incubation with CaP cells in the Transwell system, human DC expressed lower levels of costimulatory molecules CD80 and CD86 and were much less potent inducers of T cell proliferation in an allogeneic mixed leukocyte reactions (MLR) [11,12]. It was also demonstrated that CaP induced apoptosis of human and murine DC in vitro [13,14]. However, there are no data on the effect of CaP on DC generation from murine bone marrow precursors. To investigate the influence of CaP on murine DC generation, we first analyzed DC phenotype, after co-incubating DC with two different CaP cell lines (RM1 and TRAMP-C2). Next, we prepared murine CaP cells (RM1-TK-neo) transduced with Herpes simplex virus-thymidine kinase gene (HSVtk) and used these cells both for in vitro co-cultures directly with DC precursors, as well as for intrafemoral bone marrow injections, to evaluate the effect of CaP on in vivo marrow precursors. Finally, we evaluated CaP inhibition of DC generation in the transgenic adenocarcinoma of mouse prostate (TRAMP) mouse model.

**MATERIALS AND METHODS**

**Animals**

Male C57BL/6 mice, 6- to 8-week-old, were obtained from Taconic (Germantown, NY). TRAMP mice and control transgenic mice were bred at the University of Pittsburgh Animal Facility. Animals were housed in a pathogen-free facility under controlled temperature, humidity, and a 12-hr light/dark cycle with food and water available ad libitum. All animals were acclimatized for at least 2 weeks prior to the experiments.

**Tumor Cell Lines and Culture Medium**

Murine CaP cell lines RM1-wt and TRAMP-C2 were kindly supplied by Dr. T.C. Thompson and Dr. N.M. Greenberg (Baylor College of Medicine, Houston, TX). Tumor cells were cultured (37°C, 5% CO2) in complete medium (CM) (RPMI-1640, 2 mM L-glutamine, 50 g/ml gentamicin sulfate, 10 mM HEPES, 10% FBS, 10 mM non-essential amino-acids, 1 mM sodium pyruvate).

**Retroviral Vectors and Tumor Cell Line Transfection**

Producer cell lines for the retroviral vectors coding cDNAs of HSVtk and neo®, CRIP-DFG-TK-neo and coding cDNAs of GFP and neo®, CRIP-DFG-GFP-neo were prepared by the Vector Core Facility [15]. RM1-cells were transduced using a standard protocol [16] and selected for antibiotic resistance in culture medium containing 750 µg/ml, G418 (Invitrogen, Carlsbad, CA) for RM1-TK-neo and 250 µg/ml zeomycin (Invitrogen) for RM1-GFP-neo.

**DC Cultures**

Murine DC were generated from hematopoietic progenitors isolated from bone marrow [17]. Bone marrow cells were collected from femurs of mice, passed through a nylon cell strainer to remove pieces of bones and debris. Bone marrow cells were then depleted of red blood cells with lysing buffer and incubated with anti-mouse B220, CD4, and CD8 antibodies for 1 hr at 4°C followed by incubation with rabbit complement for 30 min at 37°C to deplete B and T lymphocytes. The cells were cultured (37°C, 5% CO2) in CM in 6-well plates (10⁶ cells/ml) overnight. Next, the non-adherent cells were collected, resuspended in CM supplemented with murine GM-CSF (1,000 U/ml) and IL-4 (1,000 U/ml) and cultured in 6-well plates (2 × 10⁶ cells/ml) at 37°C, 5% CO2 for 7 days with an additional supplementation of GM-CSF and IL-4 on day 4. DC were harvested, counted, and used for further analysis.

**Flow Cytometry**

DC phenotype was evaluated using flow cytometry analysis. Harvested cells were washed in FACS medium (HBSS containing 0.1% BSA and 0.1% NaN₃) and stained with appropriately diluted antibodies directly conjugated with FITC or PE according to the
standard procedure, and followed by fixation in 2% paraformaldehyde. Antibodies used for FACS staining were the following: FITC-labeled anti-mouse MHC class II, CD86, CD80, CD40, and PE-labeled CD11c (PharMingen, San Diego, CA). Fluorescence was measured using a FACScan flow cytometer (Becton Dickinson, San Diego, CA) and data analysis was performed using the Cell Quest Software (Becton Dickinson).

**MLR**

Functional activity of DC was determined in the primary allogeneic MLR assay using mouse T lymphocytes as responder cells as described earlier [17]. DC used as stimulators (H-2Kb), were generated from bone marrow progenitors of C57BL/6 mice. Allogeneic T cells (H-2Kd), obtained from the spleens of BALB/c mice, served as responders. Allogeneic T cells were obtained from spleen cell suspensions by passage through the nylon wool columns after lysing of red blood cells. The MLR assays were carried out in round-bottomed 96-well plates where DC were added in triplicates in graded doses (10^3–10^5 cells/well) to T cells (3 × 10^5 cells/well) in a total volume of 200 μL. Proliferation of T cells was measured by uptake of 3H-thymidine (1 Ci/well, 5 Ci/mmol; DuPont-NEN, Boston, MA) pulsed for 16–18 hr after 3 days in culture. The cultures were harvested on GF/C glass fiber filter paper (Whatman Intl. Ltd., Maidstone, England) using a MACH III microwell harvester (Tomtec, Hamden, CT). Incorporation of 3H-thymidine was determined on a MicroBeta TRILUX liquid scintillation counter (WALLAC, Gaithersburg, MD) and expressed as a count per minute (cpm).

**Immunohistochemical Analysis**

Splenic tissue samples from TRAMP mice were collected in OCT compound (Tissue-TEK, Mites, Elkhart, Ind., USA) and stored at −80°C. Cryostat sections (3–5 μm) were fixed in cold acetone for 15 min and washed twice for 5 min in PBS. Endogenous peroxidase activity was blocked by 0.3% hydrogen peroxide in PBS. Slides were then washed three-times in PBS and incubated for 1–2 hr at room temperature with hamster anti-mouse CD11c-specific Ab (Serotec Inc., Raleigh, NC). Biotin-labeled secondary Ab (Vector Laboratories, Burlingame, CA) were applied to the slides and incubated for 45 min at room temperature at a dilution of 1:200. After subsequent washes in PBS, slides were incubated with avidin–biotin–horse-radish peroxidase (ABC; Vector Laboratories) for 30 min at room temperature and color reaction was developed using an AEC kit. The presence of CD11c^+ cells was evident as a red–brown reaction product. Staining with normal hamster IgG was used as a negative control.

**Experimental Design InVivo**

**Transwell system.** Bone marrow cells were collected from C57BL/6 mice as mentioned above. DC precursors (2 × 10^5 cells/ml) were co-cultured (37°C, 5% CO2) with RM1 or TRAMP-C2 cells (5 × 10^4 cells/ml) or medium alone in 6-well plates separated by membrane inserts with 0.4 μm pore size in CM with GM-CSF and IL-4 for 7 days. Splenocytes were used as additional control. No significant differences were determined between DC cultures co-incubated with splenocytes or medium alone (P > 0.05). DC were harvested and their phenotype and function were analyzed.

**Mixed cell system.** To prepare gancyclovir (GCV)-sensitive CaP cell line (RM1-TK-neo), RM1 cells were transduced with HSVtk. Different numbers of RM1-TK-neo cells (10, 100, and 1,000 cells/well) were directly added to DC precursors (2 × 10^5 cells/ml) and depleted 6 days later using 1 μg/ml GCV. To separate live and dead cells, cell suspensions were loaded onto the NycoPrep solution (1:1 mixed 1.068 and 1.077 g/ml, Nycomed Pharma AS, Oslo, Norway) and centrifuged for 20 min at 400g. Live DC were collected and analyzed. The viability of DC was greater than 95%.

**Experimental Design InVitro**

A. Intra-bone marrow (intrafemoral) injection of RM1-TK-neo cells. Mice were anesthetized with methoxyflurane. RM1-TK-neo tumor cells (20 × 10^3 cells/20 μL in HBSS) or 20 μL HBSS were injected into the bone marrow in the left femur of the mice using microliter syringes (Hamilton, Reno, ND) with 27 G needles. Seven days after tumor injection, bone marrow cells were collected from the treated left femur. After removing RBC, CD4+, CD8+, and B220+ cells as described above, the rest of the cells were incubated in CM with 1 μg/ml GCV for 24 hr. The non-adherent cells were harvested, separated from dead cells, and cultured in 6-well plates (2 × 10^5 cells/ml) at 37°C, 5% CO2 for 7 days with an additional supplementation with GM-CSF and IL-4 on Day 4. DC were harvested, counted, and used for further analysis.

To evaluate how many tumor cells were still alive after intrafemoral injection RM1-GFP-neo cells were used as a control. Seven days after tumor injection (20 × 10^3 cells/20 μL in HBSS), bone marrow cells were collected and analyzed for GFP positive cells by FACScan. GFP positivity was 35.8 ± 5.6% in RM1-GFP-
poiesis in a Transwell system in vitro. CaP cell lines RM1 and TRAMP-C2 inhibit dendro-
DC precursors with CaP cells significantly decreased Flow cytometry analysis revealed that the incubation of a Transwell system and DC phenotype was evaluated. cultured in the presence of RM1 and TRAMP-C2 cells in hematopoietic precursors, bone marrow cells were maintained in a pure C57BL/6 background. TRAMP mice were bred to non-transgenic FVB mice to obtain non-transgenic and transgenic [C57BL/6TRAMP × FVB]F1 males [19]. Non-transgenic littersmates were used as controls. Bone marrow cells were collected from tibias and femurs, depleted of RBC, CD4+, CD8+, and B220+ cells as described above, and cultured (37°C, 5% CO₂) in CM in 6-well plates (10⁵ cells/ml) overnight. Non-adherent cells were collected, resuspended in CM supplement with murine GM-CSF (1,000 U/ml) and IL-4 (1,000 U/ml), and cultured in 6-well plates (2 × 10⁵ cells/ml) at 37°C, 5% CO₂ for 7 days with additional supplementation with GM-CSF and IL-4 on day 4. DC were harvested and analyzed.

**Reagents**

RPMI 1640, l-glutamine, gentamicin, HEPES, FBS, nonessential amino-acids, sodium pyruvate, and rabbit complement were purchased from Grand Island Biological Company (Gibco, Grand Island, NY); RBC lysing buffer and paraformaldehyde, obtained from Sigma (St. Louis, MO); GM-CSF and IL-4 were a gift from Schering-Plough Research Institute (Kenilworth, NJ).

**Statistical Analysis**

Statistical significance of differences was determined using the Student’s t-test and the nonparametric Mann–Whitney test. For all statistical analysis, the level of significance was set at a probability of 0.05 to be considered significant. Data are presented as the Mean ± SEM. All experiments were repeated at least twice.

**RESULTS**

**CaP Cells Inhibit DC Generation From Bone Marrow Cells In Vitro**

CaP cell lines RM1 and TRAMP-C2 inhibit dendro-
poiesis in a Transwell system in vitro. To evaluate the effect of CaP on murine DC generation from hematopoietic precursors, bone marrow cells were cultured in the presence of RM1 and TRAMP-C2 cells in a Transwell system and DC phenotype was evaluated. Flow cytometry analysis revealed that the incubation of DC precursors with CaP cells significantly decreased (P < 0.01) the percentage of CD11c (from 60.3 to 7.2 and 8.4), CD86 (from 13.2 to 4.3 and 7.6), and CD80 (from 19.2 to 10.5 and 6.8) positive cells on DC incubated with RM1 and TRAMP-C2 cell line, respectively (Fig. 1). These results suggest that CaP-derived soluble factors suppress dendropoiesis in vitro.

**CaP Cells Inhibit DC Generation From Bone Marrow Cells In Vivo**

**Inhibition of dendropoiesis in vivo.** To determine whether CaP cells affect DC precursors within the bone marrow in vivo, intrafemoral injection of tumor cells was performed and DC cultures were initiated from tumor-treated precursors followed by DC analysis of phenotype and function. Flow cytometry analysis revealed that the percentage of double-
positive CD11c/MHC II, CD11c/CD86, and CD11c/CD80 cells was reduced from 51.6 ± 5.2 to 37.3 ± 4.1,
from 40.9 ± 2.5 to 24.6 ± 2.7, and from 46.4 ± 3.0 to 38.3 ± 3.5, respectively, in vivo tumor-treated
cultures compared with non-treated DC cultures (P < 0.05). Furthermore, the allogeneic MLR assay
revealed that the ability of these DC to induce T cell proliferation was also decreased (Fig. 3). For instance,
at effector:target ratio 1:30, uptake of 3H-thymidine was reduced by 34% and at the ratio 1:300—by
47% (P < 0.05). These results suggest that murine CaP cells injected intrafemorally inhibit the ability
of bone marrow progenitors to differentiate into functional DC.

Inhibition of dendropoiesis in TRAMP mice. Analysis of DC generated in vitro from hematopoietic
precursors isolated from 18- and 24-week-old TRAMP mice revealed a progressive decrease in DC generation
determined as the percentages of double-positive CD11c/CD86 and CD11c/CD40 DC in cultures
(Fig. 4). For example, double-positive CD11c/CD86 cells decreased from 56 to 16% and 10%, and double-
positive CD11c/CD40 reduced from 43 to 6% and 2%, in control mice, 18-week-old TRAMP mice, and
24-week-old TRAMP mice, respectively (P < 0.05). Furthermore, immunohistochemical analysis of endo-
genous in vivo generated splenic DC in TRAMP mice showed a marked inhibition of CD11c+ DC in com-

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Fig. 1. Prostate cancer (CaP) cell lines RM1 and transgenic adenocarcinoma of the mouse prostate (TRAMP)-C2 inhibit dendropoiesis in vitro. Bone marrow cells were cultured (days 1–7) in the presence of RM1 and TRAMP-C2 cells in a Transwell system as described in "Materials and Methods." Dendritic cells (DC) precursors co-cultured with medium alone served as a control. FACS analysis was performed using anti-mouse FITC-labeled CD86, CD80 and PE-labeled CD11c Abs. The results of one of three representative experiments are shown.
Fig. 2. CaP RMI-TK-neo cells added directly to DC cultures suppress DC generation in a dose-dependent manner. Bone marrow cells (2 x 10^5 cells/ml) were co-cultured with different numbers of RMI-TK-neo cells (10 (△), 100 (■), and 1,000 (▽) cells/well) in CM supplemented with GM-CSF and IL-4 from days 1 to 6 followed by depletion of RMI-TK-neo cells using 1 μg/ml gancyclovir (GCV). Bone marrow cells cultured without tumor served as a control (●). On day 7 live cells (after elimination of RMI-TK-neo cells with GCV) were collected and their phenotype (A) and capacity to activate T cells in allogeneic mixed leukocyte reactions (MLR) (B) were determined. A, FACScan analysis was performed using double staining with anti-mouse FITC-labeled MHCII, CD86, CD80 and PE-labeled CD11c Abs. Data represent the mean ± SEM from three independent experiments. *P < 0.01 versus control. B, The MLR assay was performed as described in “Materials and Methods.” DC were used as stimulators, allogeneic splenic T cells served as responders. DC were added in graded doses (10^3 – 10^5 cells/well) to T cells (3 x 10^5 cells/well) and proliferation of T cells was measured by uptake of ^3H-thymidine. Data represent the mean ± SEM from three independent experiments. *P < 0.01 versus control.
parison with control tissues (Fig. 5). Splenic tissue samples were obtained from TRAMP mice at different ages and analyzed for the presence of DC based on the expression of DC marker CD11c. Numbers of splenic DC were significantly lower in TRAMP mice in comparison with control-transgenic animals of the same age. This suggests that CaP progression in mice is accompanied by inhibition of DC generation in vivo.

**DISCUSSION**

Tumor-related suppression of DC function has recently been reported as a new mechanism of tumor escape from immuno-surveillance [2,20]. For instance, human CaP inhibits the generation and maturation of human DC from CD14+ monocytes or CD34+ precursors in vitro [11,12]. Here we have shown that murine CaP inhibits murine hematopoietic DC precursors both in vivo and in vitro in different murine models of CaP.

In the in vitro model, when murine bone marrow cells were co-cultured with CaP cells in a Transwell system, we demonstrate that CaP-derived factors hampered the differentiation of hematopoietic precursors into DC. The nature of the CaP-related factors responsible for inhibition of DC function remains unclear. These factors may be specific proteins such as prostate specific antigen (PSA) and prostatic acid phosphatase (PAP), which are secreted by luminal cells and serve as markers of exocrine differentiation, or/and substances such as chromogranin A, serotonin, neuron-specific enolase, bombesin, neuromedin B, and calcitonin gene-related peptide (CGRP), which are expressed by neuroendocrine cells and serve as markers of neuroendocrine differentiation. Elevation of both exocrine and neuroendocrine markers in serum is known to be associated with CaP progression, and in general there is significant correlation between cancer progression, and/or mortality, and the level of these markers in sera from patients [21,22]. In fact, we have recently reported that both purified and CaP-derived PSA could significantly inhibit both murine and human dendropoiesis in vitro [23]. These data are also consistent with our previous results demonstrating inhibition of generation of human DC from CD14+ monocytes by neuropeptides derived from lung cancer cell lines [24]. There are also several reports describing the inhibitory effects of soluble tumor-derived factors such as IL-10, IL-6, VEGF, TGFβ, and gangliosides on DC generation and function [20,25–27]. In addition, it has recently been shown that

![Fig. 3. Intrafemorally injected CaP cells inhibit DC ability to induce T cell proliferation in an MLR assay.](image-url)
cyclooxygenase-regulated prostanoids, which are present in primary tumor-derived supernatants from freshly excised human tumors (colon, breast, renal cell carcinoma, and melanoma) profoundly hamper DC generation from both CD14$^+$ monocytes, as well as from CD34$^+$ precursors in vitro [28]. Collectively, these reports lend credence to the fact that tumor-derived soluble factors from several different cancers (including CaP), are responsible for suboptimal DC generation and differentiation, and this impairment of DC function may represent a novel mechanism of tumor escape from immuno-surveillance.

To determine the effect on dendropoiesis of directly adding tumor cells to bone marrow precursors, we prepared murine CaP cells (RM1-TK-neo) transduced with the HSV$^{tk}$ and then co-cultured them with DC in vitro. In separate experiments, the RM1-TK-neo cells were also directly injected intrafemorally, to evaluate the effects of these cells on in vivo DC generation. The in vitro TK-neo-transduced RM1 cells were directly added to DC cultures and eliminated later using GCV. A dose-dependent inhibition of DC generation and maturation confirmed that murine CaP cells inhibit the ability of hematopoietic progenitors to differentiate into functionally active DC in vitro. Similarly, the intrafemorally injected RM1 cells also suppressed marrow hematopoietic precursors and the ability to generate DC from these precursors.

Inhibition of hematopoiesis is a common feature in patients with cancer [29,30]. Since CaP has a propensity for metastasizing to bone, the suppressive effect of CaP on hematopoiesis may significantly increase with worsening bone marrow metastasis, as there is direct contact between tumor cells and hematopoietic precursors. The blocking of DC generation in the bone marrow by metastasizing CaP cells maybe one of the reasons for the marked decrease in tumor-infiltrating DC in advanced CaP. While CaP is well known to commonly metastasize to the bone [31,32], there are no reproducible and common experimental models to

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**Fig. 4.** Inhibition of DC generation in vitro from bone marrow precursors obtained from TRAMP mice. DC were generated in cultures from bone marrow-derived hematopoietic precursors obtained from 18- and 24-week-old TRAMP mice as described in "Materials and Methods." FACScan analysis was performed using double staining with anti-mouse FITC-labeled CD86, CD40 and PE-labeled CD11c Abs. The results of one of three representative experiments are shown.

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study the effect of syngeneic metastasis on bone marrow DC precursors in mice. A few bone xenograft models for CaP metastasis using intrafemoral injection of LNCaP, C4-2, LNCaP-C4-2, or PC-3 cell lines in athymic or SCID/bg or Balb/c nude mice have been tested [33–35]. However, these xenograft models are not suitable for characterizing the effects of CaP on DC generation, given the difficulty in evaluating the immune response in nude mice. Additionally, it is also difficult to separate bone marrow precursors from tumor cells, a problem which we have circumvented, by transducing the RM1 cell line with TK. Thus, using intrafemoral injections of transduced CaP cell line RM1-TK-neo and GCV treatment, we have developed a syngeneic tumor bone marrow invasion model, which maybe useful in studying the direct effect of CaP (RM1 cells) on bone marrow hematopoietic precursors in vivo.

Finally, we were also able to demonstrate CaP-induced inhibition of DC generation in TRAMP mice. The TRAMP model has received considerable attention over the past several years. The model was generated with a PB-Tag transgene containing the SV40 early genes (T and t antigens, Tag) under the control of the minimal rat probasin (PB)—426/+28 promoter [36]. TRAMP mice demonstrate spontaneous genomic instability, reduced p53 dependent apoptosis, and mimic the consequence of constitutive growth factor signaling. By 12 weeks of age TRAMP mice histologically display mild to severe hyperplasia with cribiform structures that progress to severe hyperplasia and adenocarcinoma [37]. The TRAMP model has been thoroughly characterized and tested with a number of therapeutic pre-clinical trials, including immunotherapy trials [38]. In this study, we have shown that progressive development of CaP in TRAMP mice was

Fig. 5. Progressive decrease in numbers of splenic CD11c+ DC in TRAMP mice in vivo. Splenic tissues were obtained from 18- and 24-week-old TRAMP mice or control transgenic mice (control) and DC were assessed by immunohistochemical staining with anti-CD11c antibody (red staining). Tissues were counterstained with hematoxylin and eosin. The results of one of three representative experiments are shown.
accompanies a progressive inhibition of dendro-
poiesis. We have demonstrated a significant inhibition of
dendropoiesis both in vitro and in vivo in TRAMP mice.

In conclusion, we have analyzed how CaP effects on
differentiation of hematopoietic precursors into DC inoth in vitro and in vivo murine models. Suppression of
DC generation together with tumor-induced apoptosis and
progressive inhibition of DC function maybe an
important mechanism, whereby CaP escapes immune
recognition and elimination. Elucidating the mechan-
isms responsible for the inhibition of DC generation
and function in the CaP microenvironment may result in
the development of strategies for circumventing these
defects in DC function, and ultimately aid in the design
of more efficacious DC based vaccines for the treatment
of patients with CaP.

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