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Activation and Protection of Dendritic Cells in the Prostate Cancer Environment

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14. ABSTRACT
Final Report for research performed at the UMDNJ and VCU. Experiments have demonstrated for the first time the presence of endothelin receptors on murine dendritic cells (DC), and the fact of endothelin-1 production by murine DC upon stimulation with TNF• and lipopolysaccharide (LPS). The modification of the endothelin axis on DC changed its resistance against prostate cancer induced apoptosis – the blockade of ET_A receptors resulted in the increased apoptotic rate, while the blockade of the ET_B receptors lead to the increased survival of DC in the prostate cancer environment. Blockade of ET_A receptors also resulted in decreased DC capability to express costimulatory molecules and promote T cell proliferation. Based on these data, in vivo experiments were carried out, in which mice with prostate cancer (RM1 cells) were treated with intratumoral injection of modified DC (stimulated DC, with ET_B receptor blocked). This treatment resulted in reduction of prostate cancer growth in mice in the experimental group, in comparison to untreated control mice. Blockade of ET_B receptors and pulsing of DC with tumor antigen also led to the reduction of tumor growth after subcutaneous injection of this vaccine. Studies are under way to elicit the mechanisms of endothelin axis action on DC, as well as the underlying mechanisms of interaction between DC and prostate cancer cells.

15. SUBJECT TERMS
Prostate cancer, Dendritic cells, Endothelin receptors, Immunotherapy

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Introduction:

This study is being conducted for the (i) characterization of the prostate cancer and dendritic cells (DC) interaction; (ii) defining the role of endothelin axis in the maturation of DC, (iii) elucidating the role of endothelin axis in the prostate cancer-DC interaction, and (iv) modification of dendritic cells to be used in the treatment of prostate cancer. Mouse model was used. This is the final report for the award, from 2005 to 2010, covering the work done at the New Jersey Medical School and at the Virginia Commonwealth University (VCU) School of Medicine. In August of 2008, I have accepted a position of the associate professor, moved the lab there and was able to complete the proposed project.

Body of the Report:

First task was to proceed with the characterization of role of endothelin axis in DC. For this purpose, DC were grown from C57BL/6 mice bone marrow, as was described earlier [1]. Briefly, bone marrow cells were first depleted of RBC with lysing buffer for 2–3 min. The single-cell suspensions then was incubated with a cocktail of Abs (αCD4, αCD8a, and B220) for 1 h at 4°C, followed by incubation with rabbit complement for 30 min at 37°C to deplete cells expressing lymphocyte antigens B220, CD4, and CD8. Cells were then incubated overnight (37°C, 5% CO_2) in six-well plates (Falcon, Franklin Lakes, NJ) at a concentration of 10^6 cells/ml in complete medium, consisting of RPMI 1640, 2 mM L-glutamine, 50 μg/ml gentamicin sulfate, 10 mM HEPES, 10% FBS, 0.1 mM nonessential amino acids, and 1 mM sodium pyruvate (Life Technologies). The nonadherent cells were collected by gentle pipetting and resuspended at a concentration of 2.5×10^5 cells/ml in complete medium supplemented with 1000 U/ml recombinant murine GM-CSF and recombinant murine IL-4 (R&D system). Cells were cultured in six-well plates (4 ml/well) for 7 days at 37°C in 5% CO_2. Nonadherent DC are collected by gentle pipetting, counted, characterized as described previously [2], and used for further studies.

For the characterization of the general impact of endothelin receptors, dendritic cells were stimulated with TNFα and lipopolysaccharide (LPS, Sigma-Aldrich) for the endothelin production and the expression of endothelin receptors, since our preliminary data indicated increased expression of endothelin receptors upon stimulation in mice (unpublished data). We have previously demonstrated as well increased production of endothelin-1 (ET-1) by human DC, and increased expression of endothelin receptors [3].

For the characterization of ET-1 production, DC were cultured as described above, and stimulated with TNFα (10ng/ml, added on Day 5) or LPS (200ng/ml, added on Day 5) for 48 hours. After that, cell-free supernatants were collected, and ET-1 concentrations were measured using ET-1 enzyme-linked immunosorbent assay (ELISA) system (R&D systems, Minneapolis, MN); in every assay, samples and standards were run in duplicates and read at 450-nm wavelength on a microplate reader. ET-1 concentrations were normalized based on cell counts and determined by computer software–generated interpolation from the standard curve.

As it can be seen on Figure1, control DC (unstimulated) produced 22.67±2.34 pg/ml/mln cells. TNFα stimulated cells produced 67.35±6.92 pg/ml/mln cells (The difference was statistically significant, $P<0.001$), and LPS-stimulated celles produced

84.24±3.84 pg/ml/mln cells (The difference was statistically significant – $P<0.001$, in comparison to control. There was no statistically significant difference in ET-1 production levels among cells stimulated with TNFα or LPS).

Next, we evaluated the expression of the endothelin receptors upon stimulation. DC were cultured as usual, and stimulated with TNFα and LPS as described above. Cells were collected on day 7, air dried and fixed in methanol at -20°C for 20 min. After the slides are washed with PBS, they are blocked in 10% normal goat serum (1 hour in room temperature) and incubated with antibodies against ET_A or ET_B receptors (1:50 dilution, Alomoni Labs, Israel) at 4° C overnight. Following PBS wash (5 min x 2), the cells were incubated with fluorescent secondary antibody at room temperature for 1 hour. Immunostaining of ET receptors was examined with a Nikon fluorescent microscope. The results are presented on Figure 2. As it can be seen, the the stimulation of DC indreased the expression of endothelin receptors.

Changes in phenotype have been evaluated as well. Murine DC has been stimulated with TNFα on day 5 for 48 hours. Stimulated DC were treated with endothelin receptor inhibitors BQ-123 (Selective ET_A receptor inhibitor, American Peptide Company), at a final concentration of 10^{-6} *M*, for the last 48 hours, and with BQ-788 (Selective ET_B receptor inhibitor, American Peptide Company), at a final concentration of 10^{-6} *M*, for the last 48 hours as well. After that, cells were collected, washed, counted and stained for flow cytometry. We have evaluated cells for the expression of CD40, CD80, CD86, MHC class II antigen, and CD205. Briefly, stimulation with TNFα resulted in the increased expression of these costimulatory molecules (as expected). The blockade of ET_A receptor with BQ-123 induced in general decreased expression of the costimulatory molecules, which was especially significant for CD40 and CD205 (difference was statistically significant by chi-square test, $P<0.001$). On the other hand, the blockade of ET_B receptor with BQ-788 resulted in no change or increased expression of costimulatory molecules (Figure 3).

For the further characterization of the endothelin axis on dendritic cells, we proceeded with the mixed leukocyte reaction (MLR). Briefly, allogeneic T cells were generated from balb/c mice spleens using murine T cell enrichment columns (R&D Systems, Minneapolis, MN). Isolated T cells were placed in the round-bottom 96-well plates, $3x10^5$ per well, and DC were added with decreasing concentrations. After 72 hours of incubation at 37°C, ^{3}H-TdR (New England Nuclear Co., Boston, MA) was added to the DC/T cell mixture, 1μCi per well. T cell proliferation was measured by the ^{3}H-TdR uptake in 16 hours. Cells were harvested onto glass fiber filter paper with a semi-automated microharvester, and ^{3}H-TdR incorporation was determined by liquid scintillation spectroscopy. There were four experimental groups: 1) DC prepared as usual; 2) DC treated with TNFα during the last 48 hours; 3) DC treated with TNFα and BQ-123 for the last 48 hours, and 4) DC treated with TNFα and BQ-788 for the last 48 hours. Preliminary results of the experiment are presented in the Figure 4. As it can be seen, addition of TNFα resulted in increased ability of the DC to stimulate T cells. While the addition of the BQ-788 didn't produce significant changes in these preliminary experiments, addition of BQ-123 resulted in decreased ability of DC to stimulate T cells, in comparison to DC treated with TNFα alone. These results lead us to speculate that the stimulation of ET_A receptors may lead to the activation of DC, and that their blockade might abolish or lessen immune response.

During the next set of experiments, the influence of prostate cancer cells on DC was evaluated. DC were grown as described above. On days 5 and 6, 1 ml of RM-1 (murine prostate cancer cells) cells supernatant was added to each well (in the 6-well plates). Control DC received media only (1 ml per well on days 5 and 6). On day 7, cells were collected, and flow cytometry was performed to evaluate for the expression of costimulatory molecules. Results are presented on Figure 5. Addition of tumor cells supernatant resulted in the 10% drop in the expression on CD80 marker, the expression of CD205 was reduced significantly as well.

Next, we evaluated the influence of endothelin receptors on DC survival in the prostate cancer environment. As we have already demonstrated previously [3], blockade of ET_A receptors worsened DC survival, while the blockade of ET_B receptors improved DC resistance against apoptotic stimuli. Now, in mice model, we evaluated the influence endothelin axis modification on DC survival in the prostate cancer environment. We performed the incubation of murine DC with RM-1 cells (murine prostate cancer cells), which resulted in DC apoptosis (apoptotic rate 30.96±3.9%). Pretreatment of DC with TNFα lowered apoptotic rate to 25.37±2.7%. Blockade of ET_A receptors with BQ-123 increased prostate cancer-induced DC apoptosis to 45.45±4.6%. Blockade of ET_B receptors with BQ-788 improved DC resistance to prostate cancer-induced apoptosis and dropped apoptosis rate to 16.90±3.3% (Figure 6).

In vivo experiments were performed as well. Tumors were induced by subcutaneous injection of 25,000 RM-1 murine prostate cancer cells into groups (n=5) of the C57BL/6 mice. When tumors became palpable (day 6), treatment was initiated with injection of $1.5x10^6$ bone marrow derived DC into the tumor. Group 1 received Hank's solution (control); Group 2 – unmodified DC; Group 3 –DC treated with TNFα during the last 48 hours; Group 4 - DC treated with TNFα and ET_B receptor antagonist BQ-788 during the last 48 hours. Two further DC injections were performed on days 9 and 12. Tumor volume was assessed by measuring the perpendicular tumor diameters with a Vernier caliper (Electron microscopy Sciences, Ft. Washington, PA). Tumor volume was calculated using the formula of rotational ellipsoid: m_1^2 X m_2 X 0.5236, where m_1 represents the shorter axis and m_2 the longer axis [4]. Tumor size was assessed starting from day 14 until animal sacrifice. By day 24, mean tumor volume reached 1796±166 mm^3 in the Group 1 (control), 1556±186 mm^3 in the Group2, 1508+166 mm^3 in the group 3, and 397±186 mm^3 in Group 4 ($P<0.001$ versus control). Difference in mean tumor size became significant starting from day 20 (figures 7 and 8).

As it can be seen from previous paragraph, direct injection of DC into the tumor proved successful. Translating these findings into clinical trials, that will mean the injection of modified dendritic cells into the tumor (in our case, in the prostate), which is feasible in some cases, but might not be an option in patients who had disease progression after radical prostatectomy (in which prostate is removed). To address the need of these patients as well, we scheduled a new set of experiments, which included subcutaneous injection of the modified DC away from the tumor. by manipulating the endothelin receptors, and DC stimulation with tumor antigens. TNFα was used for DC maturation and to increase the expression of endothelin receptors, while ET_B receptors where blocked by BQ-788. This combination should mature DC, made them more resilient to prostate cancer-induced apoptosis, and hopefully increase the expression co-stimulatory pro-inflammatory molecules. RM-1 cell lysates were used as antigens to

provide activated DC specific target. Lysates were obtained by repeated freeze/thaw cycles of the RM-1 cells (total of 5 cycles). Tumors were induced by subcutaneous injection of 25,000 RM-1 murine prostate cancer cells into groups of the C57BL/6 mice. Date of tumor injection was considered as day 0. There were 5 mice per group, and a total of 5 groups. Treatment was started on day 5, and repeated on days 10 and 14. Injections were performed in the flank opposite to tumor cells injection.

Group 1 received Hank's solution (control); Group 2 – DC with TNFα; Group 3 – DC stimulated with RM-1 cell lysates (20 μg/ml) during the last 48 hours; Group 4 – DC treated with TNFα (20ng/ml) and ET_B receptor antagonist BQ-788 (final concentration – 10^{-6} M) during the last 48 hours; Group 5 – DC treated with TNFα and BQ-788 and stimulated with RM-1 cell lysates for the final 48 hours. Treatment groups consisted of five mice per group. Experiment was repeated 3 times. Mice were sacrificed when they exhibited signs of distress or when total tumor volume exceeded 3000 mm^3. By day 28, mean tumor size reached 1824.08±229.86 mm^3 in the Group 1 (control), 1845.42±302.34 mm^3 in the Group 2, 1502.67±367.13 mm^3 in the Group 3, 1400.16±188.88 mm^3 in Group 4, and 922.58±90.86 mm^3 in Group 5. As it can be seen from figures 9 and 10, mice treated with modified and stimulated DC (Group 5), had the smallest tumors by the end of experiment. There was statistically significant difference in tumor sizes between Group 5 and 1 (control) as per t test (P=0.002).

Though it was not the part of the original proposal, the finding that ET-1 and its receptors were involved in the modification of immune response, prompted us to evaluate the role of endothelin axis in transplantation. Since the blockade of ET_A receptors induced increased DC apoptosis, decreased ability to stimulate T cells in mixed leukocyte reaction, and decreased expression of co-stimulatory molecules, we proceeded to study the effectiveness of ET_A receptor blockade in transplantation. There were 2 groups – control (injected intraperitoneally with water), and experimental (injected intraperitoneally with ET_A receptor inhibitor BQ-123, 5mg/kg, in 100μl of water). There were 5 mice per group, and experiment was repeated 3 times. Injections was performed for 10 days, and skin transplantation was carried out on day 5. Balb/c mice provided recipients, and C57BL/6 mice – allogeneic donors. Approximately $1cm^2$ skin was transplanted under anesthesia in the flank area. Transplanted skin was covered with bacitracin ointment and Vaseline gauze. Dressings were removed after 48 hours and skin was inspected daily after that. Graft rejection was scored on the day of total necrosis of the donor skin accompanied by beginning of scar formation on the recipient [5] (Figure 11). Results are expressed as median survival time (in days) ± standard error of the mean (SEM). Skin graft survived for 11.0±0.7 days in control group and 15.8±1.1 days in the group treated with BQ-123. Difference between groups was statistically significant (P<0.001) according to *Mann-Whitney U-test*.

One gene array experiment was performed, to assess the influence of prostate cancer cells on DC. Briefly, 7-day-old cultured DC were harvested and co-incubated with the murine prostate cancer cell line RM-1 in six-well plates. DC and tumor cells were separated using membrane inserts with 0.4-μm pore size, which exclude direct cell-to-cell contact, but allow free exchange of soluble factors. Specifically, 5 x 10^5 DC were placed in six-well plates in 3 ml of medium. One million prostate cancer cells resuspended in 2 ml of medium were placed into the inserts on the top of each well. As controls, DC were coincubated with murine splenocytes. DC were harvested 48 h later, washed, RNA was

extracted using RNA extraction minikit, and used for gene arrays. We used mouse 22K Oligo Arrays (Center for Applied Genomics) which is composed of fifteen-thousand 70 mer oligonuclotides corresponding to specific mouse transcripts. The oligonucletides were spotted onto poly-lysine-coated glass microscope slides by using a Gene Machines Omnigrid 100 arrayer (Genomic Solutions, Ann Arbor, Mich.) and SMP3 pins (Telechem, Sunnyvale, Calif.). RNA labeling and hybridization was performed using the 3DNA Array detection 350 Kit (Genisphere Inc.) according to the manufacturer's instructions. We used "comparison design" for this experiment, were RNA's were compared to each other directly, without standard. Preliminary analyze of data demonstrated so far decreased expression of receptors for IL-12 and interferon gamma in DC incubated with RM-1 cells. More experiments with "reference settings" are scheduled.

Key research Accomplishments:

- Production of ET-1 by murine DC has been documented first time, as well as the presence of endothelin receptors on murine DC upon their activation.

- The influence of endothelin receptor inhibitors on DC phenotype was demonstrated. Functional experiments (MLR) showed the possible involvement of the ET_A receptors in the activation of DC, driving them towards TH1 response. It seems that ET_B receptor stimulation might drive DC toward tolerance, with decreased expression of co-stimulatory molecules. Further studies are needed to clarify the exact role of these receptors in DC biology. Molecular biology experiments are scheduled to clarify the mechanisms of endothelin axis function in DC.

- We have demonstrated for the first time that the modification of endothelin axis on dendritic cells may result in increased resistance and improved survival against prostate cancer cells.

- Treatment of murine prostate cancer by intratumoral injection of the modified dendritic cells resulted in the reduction of the tumor growth. So did the treatment with subcutaneous injection of activated and antigen-pulsed DC. These data may provide basis for the development of clinical trials protocol.

- It has been shown first time that the modification of endothelin axis on dendritic cells (blockade of ET_A receptors) might alter immune response and prolong graft survival.

Reportable outcome:

Research data have been presented at the Annual meetings of the American Association for Cancer Research (AACR), and American Urological Association (AUA), as well as at the IMPaCT Meeting (September 5-8, 2007, Atlanta, GA). The Abstract of the presentation at the AUA is attached. Manuscript describing the major findings of the conducted research is in preparation and will be submitted in several weeks.

Conclusion:

Prostate cancer is the most common cancer in American men, and more than 32,000 are expected to die from it in this year [6]. There is now curative treatment once disease gets beyond prostate. Hormonal therapy, though efficient, is temporary and not curative, and its principle has not been changed for more than 50 years. Chemotherapy also provides only temporary and short-lived effect. Because of this, the search of alternative treatment options are vitally required. Immunotherapy based on antigen presenting cells did provide some relief to patients with hormone-resistant prostate cancer [7]. However, there is still a long way to go for it to become really effective, and large amount of work needs to be done elucidate the mechanisms of immuneotherapy and make it efficient. Our experiments have demonstrated the possible role of endothelin receptor inhibitors in the function of DC, which can be useful in the treatment of different diseases, ranging from cancer to transplantation. Our in vivo experiments showed the effectiveness of endothelin axis modification on DC in the treatment of prostate cancer in mice. More experiments are scheduled to elucidate the finer mechanisms of DC-prostate cancer interaction, and clinical trials protocol is being planned for patients with advanced prostate cancer, using modified autologous DC.

References

1. Pirtskhalaishvili G, Shurin GV, Gambotto A, et al. Transduction of dendritic cells with Bcl-xL increases their resistance to prostate cancer-induced apoptosis and antitumor effect in mice. Journal of Immunology. 2000;165:1956-1964.
2. Shurin MR, Pandharipande PP, Zorina TD, et al. FLT3 ligand induces the generation of functionally active dendritic cells in mice. Cell Immunol. 1997;179:174-184.
3. Guruli G, Pflug BR, Pecher S, Makarenkova V, Shurin MR, Nelson JB. Function and survival of dendritic cells depend on endothelin-1 and endothelin receptor autocrine loops. Blood. 2004;104:2107-2115.
4. Janik P, Briand P, Hartmann NR. The effect of estrone-progesterone treatment on cell proliferation kinetics of hormone-dependent GR mouse mammary tumors. Cancer Res. 1975;35:3698-3704.
5. McKisic MD, Macy JD, Jr., Delano ML, Jacoby RO, Paturzo FX, Smith AL. Mouse parvovirus infection potentiates allogeneic skin graft rejection and induces syngeneic graft rejection. Transplantation. 1998;65:1436-1446.
6. Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. CA Cancer J Clin. 2010;60:277-300.
7. Kantoff PW, Higano CS, Shore ND, et al. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. N Engl J Med. 2010;363:411-422.

Appendices:

Figure 1.
Assesment of ET-1 production DC. Dendritic cells were cultured as usual, and stimulated during the last 48 hours either by TNFα or LPS. Supernatants were collected and ET-1 level was measured using ELISA test. Control DC (unstimulated) produced 22.67±2.34 pg/ml/mln cells. TNFα stimulated cells produced 67.35±6.92 pg/ml/mln cells (The difference was statistically significant, P<0.001), and LPS-stimulated cells produced 84.24±3.84 pg/ml/mln cells (The difference was statistically significant – P<0.001, in comparison to control. There was no statistically significant difference in ET-1 production levels among cells stimulated with TNFα or LPS).

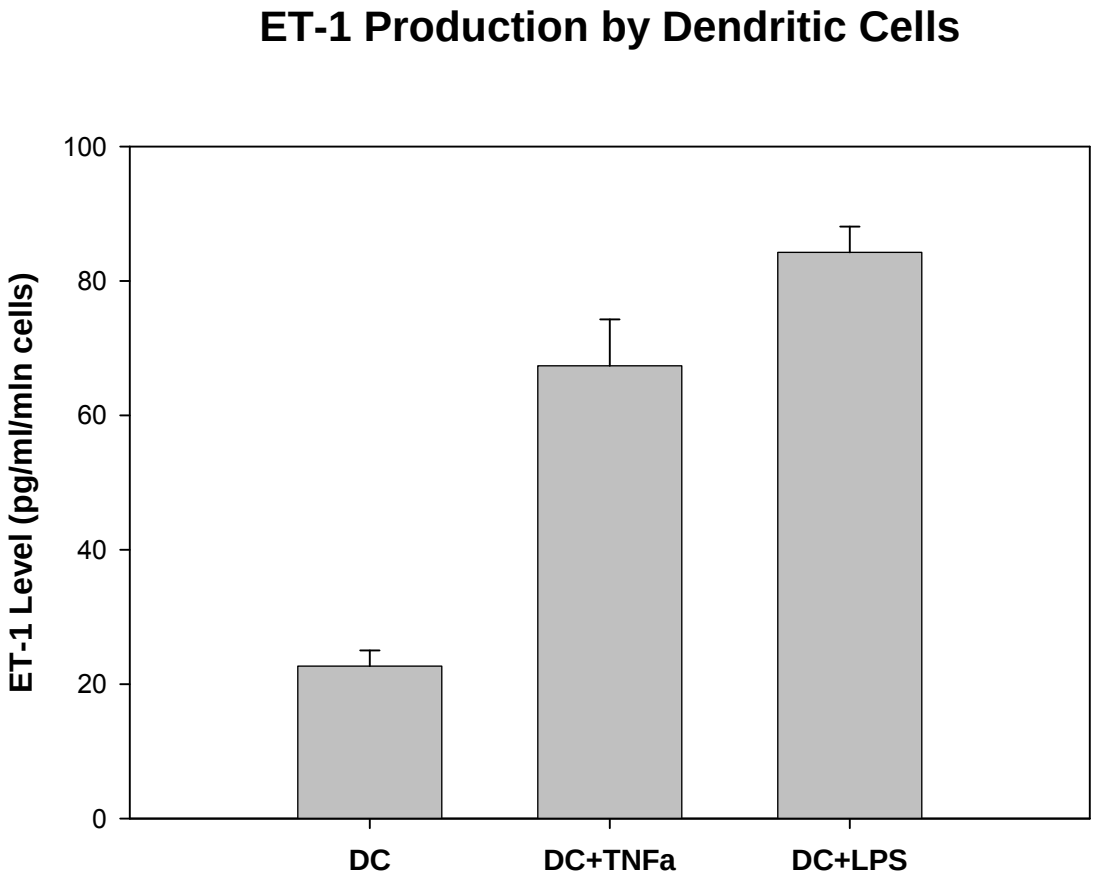


Figure 2.
Increased expression of endothelin receptors on murine dendritic cells after stimulation.
A and B, stained with ET_A antibodies,
C and D, stained with ET_B antibodies.
A and C, Unsimulated (control) DC.
B and D, Stimulated (with LPS, as described in the text) DC.

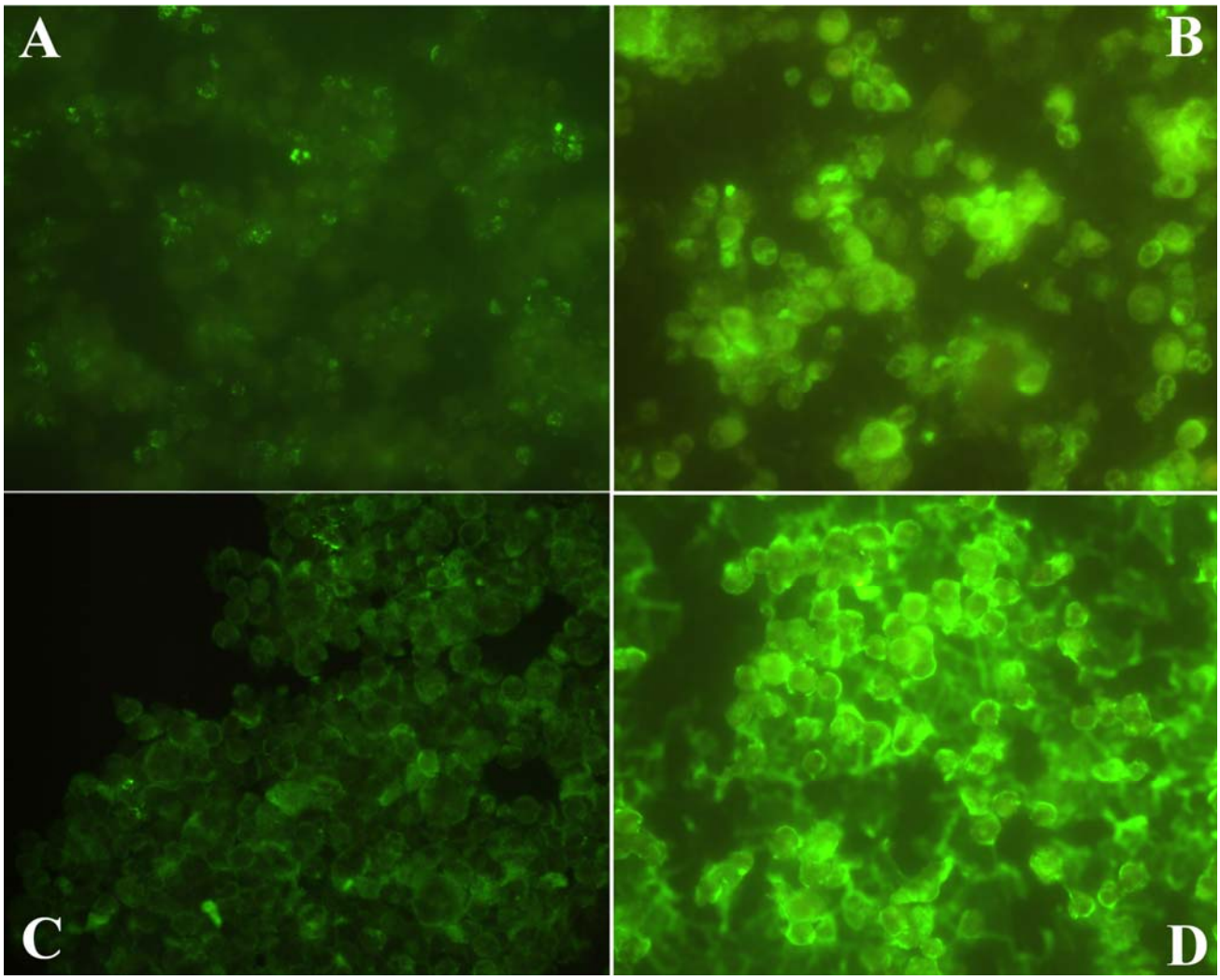


Figure 3.
Phenotyping of murine dendritic cells (DC) stimulated with TNFα and treated either with ET_A receptor inhibitor (BQ-123) or ET_B receptor inhibitor (BQ-788). Blockade of ET_A receptors resulted in the decrease of the costimulatory molecule expression, while ET_B receptor blockade was accompanied by mild increase in the expression of costimulatory molecules.

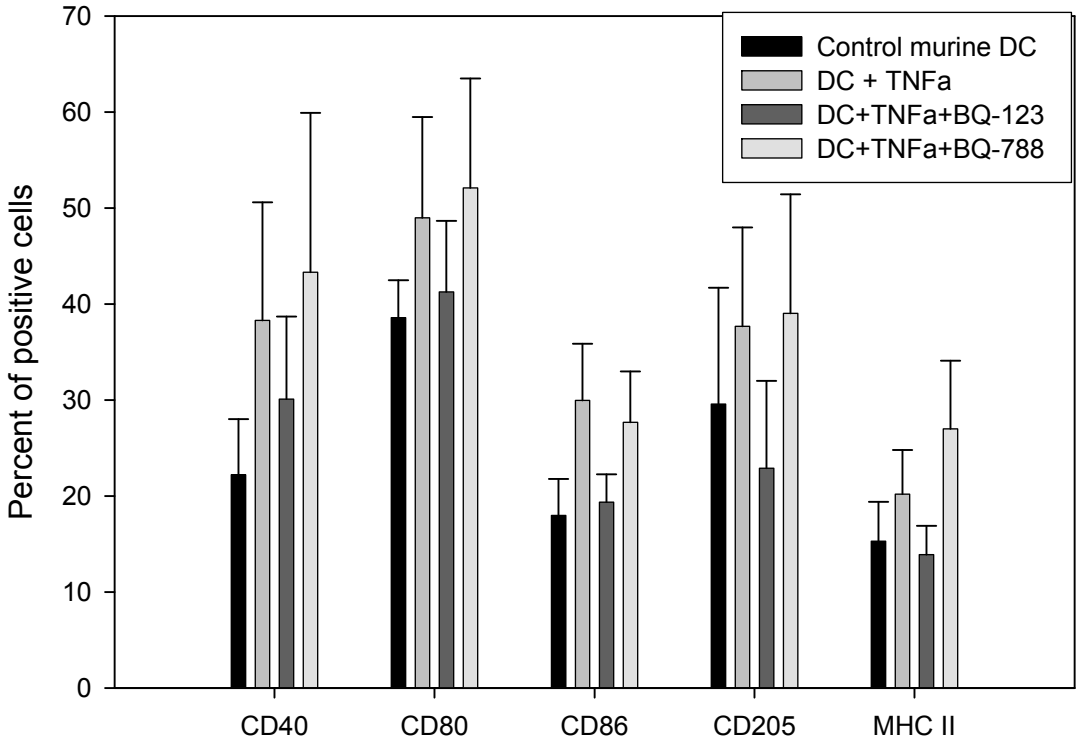


Figure 4.
The influence of the endothelin receptor inhibitors on DC is shown. Briefly, DCs were treated either with TNFα, with TNFα+BQ-123 (ET_A receptor inhibitor), and with TNFα+BQ-788 (ET_B receptor inhibitor). Untreated DC provided control. Modified DC were used to stimulate T cells in the mixed leukocyte reaction.

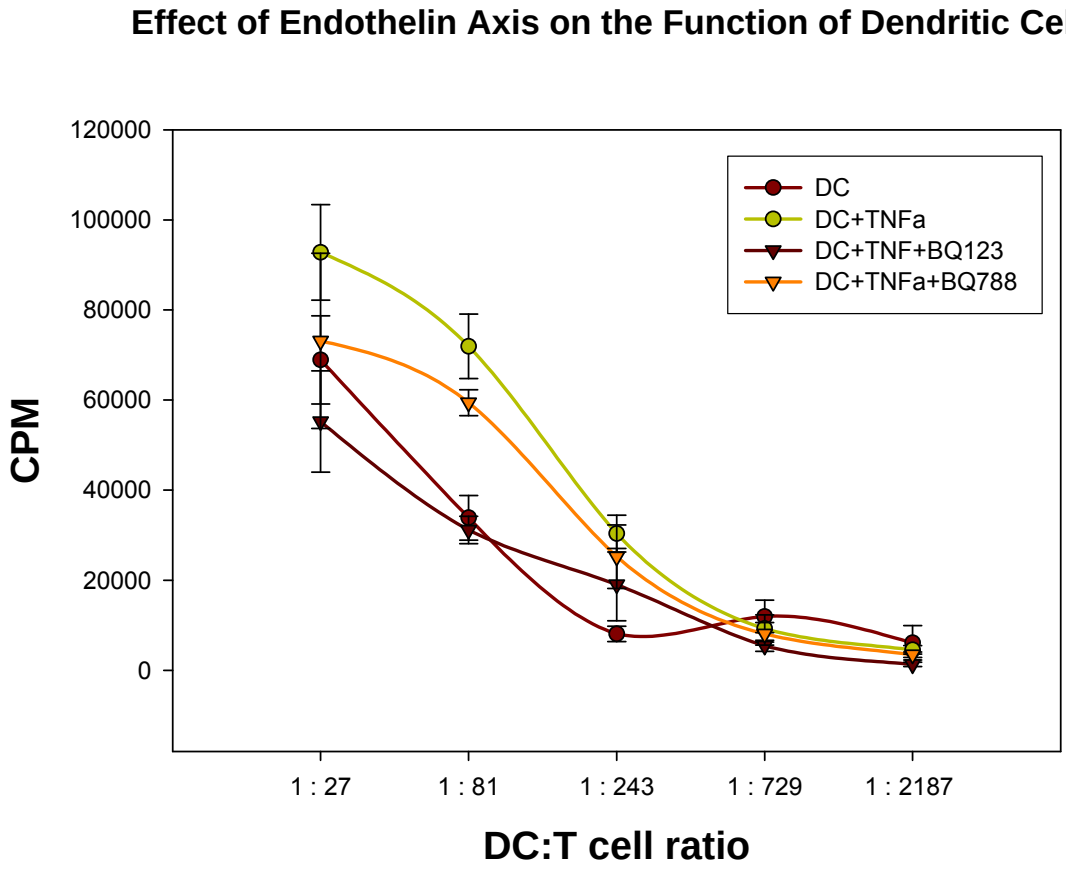


Figure 5.
Dendritic cells were grown in the culture as usual. On days 5 and 6, 1 ml of RM-1 cells (murine prostate cancer cells) supernatant was added to the culture. Control DC received 1 ml of media. Cells were collected on day 7 and flow cytometry was performed for the expression of co-stimulatory molecules.

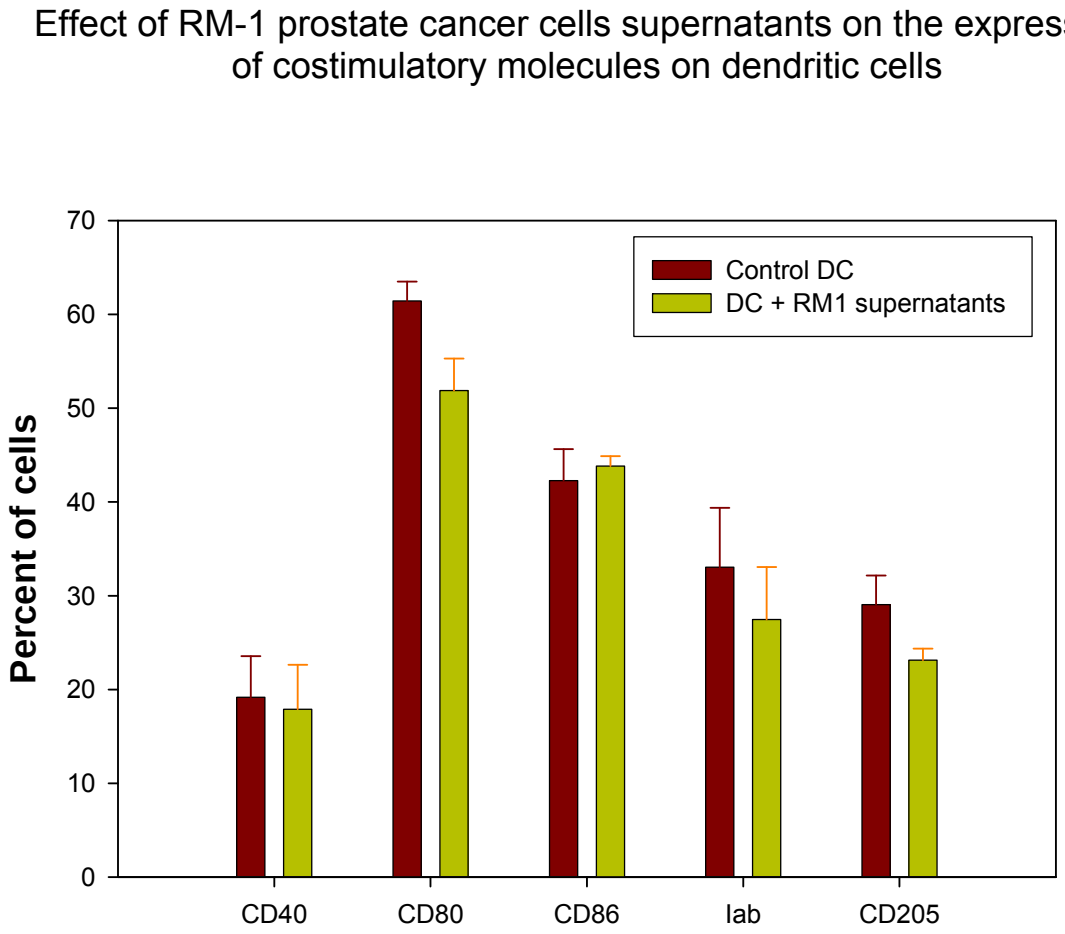


Figure 6.
Protection of dendritic cells from prostate cancer-induced apoptosis. Incubation of murine DC with RM-1 cells (murine prostate cancer cells) resulted in DC apoptosis (apoptotic rate 30.96±3.9%). Pretreatment of DC with TNFa lowered apoptotic rate to 25.37±2.7%. Blockade of ETA receptors with BQ123 increased prostate cancer-induced DC apoptosis to 45.45±4.6%. Blockade of ETB receptors with BQ788 improved DC resistance to prostate cancer-induced apoptosis and dropped apoptosis rate to 16.90±3.3%. Data of representative experiments are presented.
A – DC + RM-1
B – DC + TNFα + RM-1
C – DC + TNFα + BQ-123 + RM-1
D – DC + TNFα + BQ-788 + RM-1

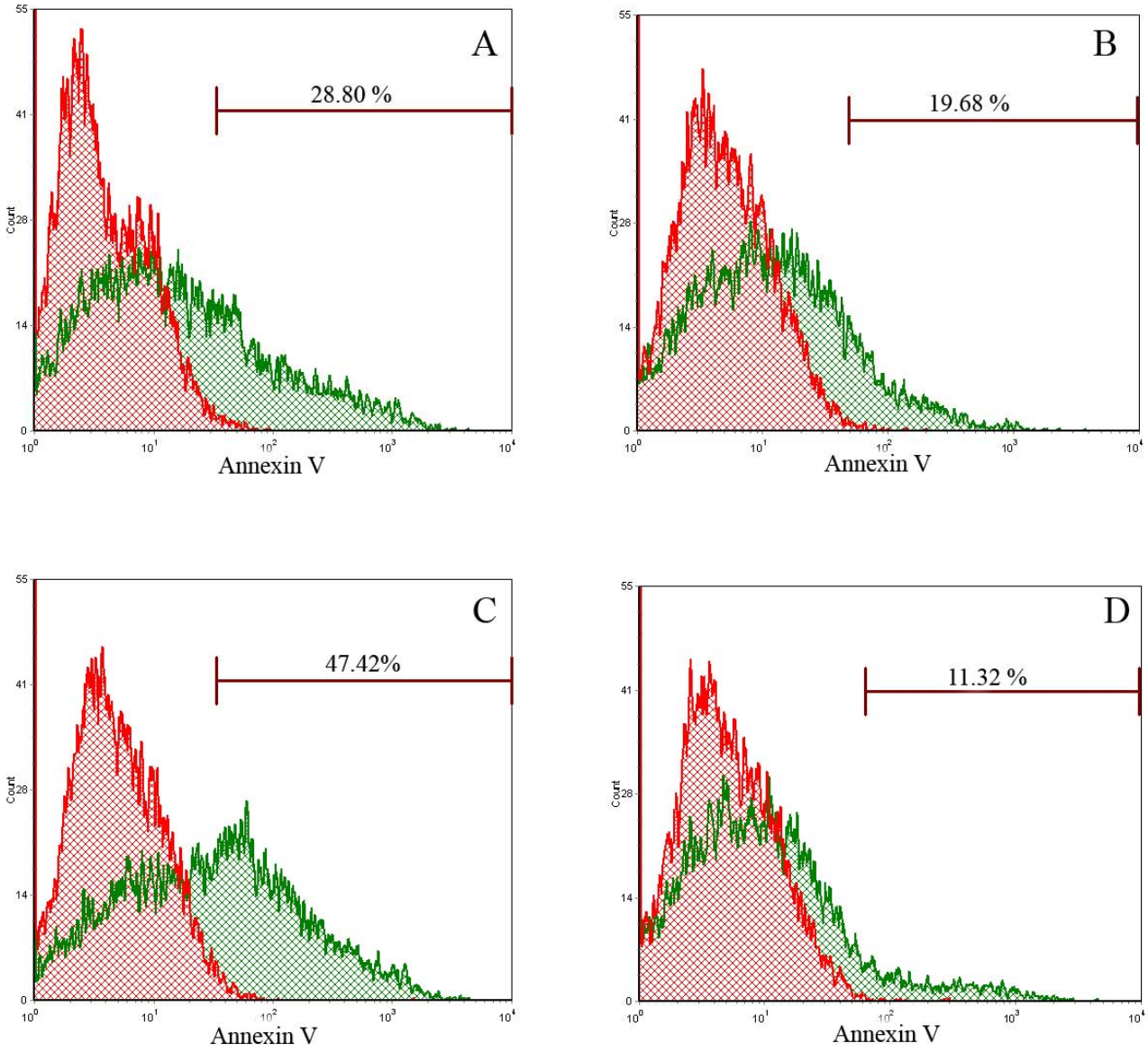


Figure 7.
Mice were injected with RM-1 murine prostate cancer cells. Treatment was started on day 6 by intratumoral injection of the dendritic cells on day 5. Group 1 (control) mice were injected with the vehicle (HBSS), Group 2 - with unmodified DC, Group 3 - with DC treated by TNFα, and group 4 - treated with TNFα and BQ-788 (ET_B receptor inhibitor). Tumor size was measured twice a week.

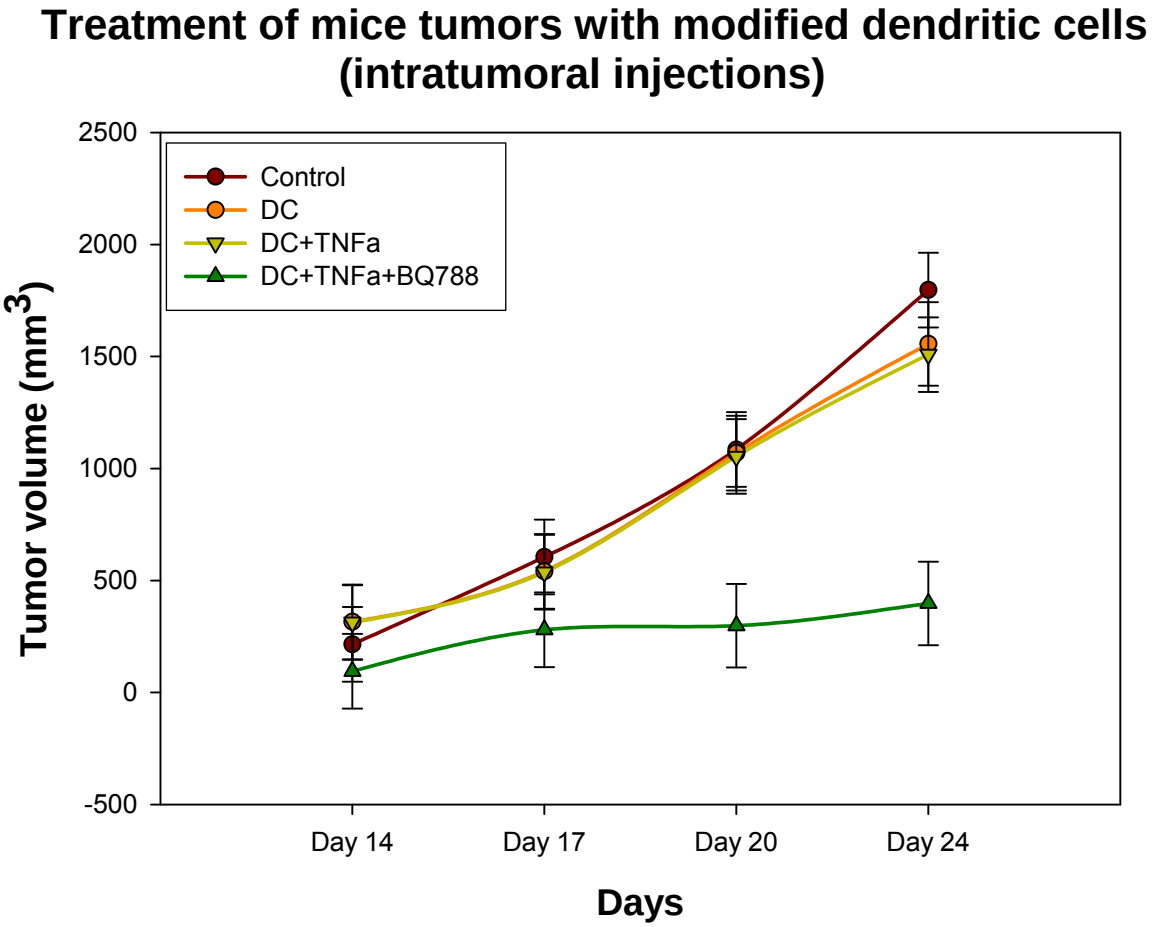


Figure 8.
Mice were injected with RM-1 murine prostate cancer cells. Treatment was started on day 6 by intratumoral injection of the dendritic cells on day 5. Group 1 (control) mice were injected with the vehicle (HBSS), Group 2 - with unmodified DC, Group 3 - with DC tretaed by TNFα, and group 4 - treated with TNFα and BQ-788 (ET_B receptor inhibitor). Tumor size was measured twice a week. Pictures of representative mice are presented. A – treated with HBSS, B – treated with DC, C – treated with DC+TNFα, D – treated with DC+TNFα+BQ-788.

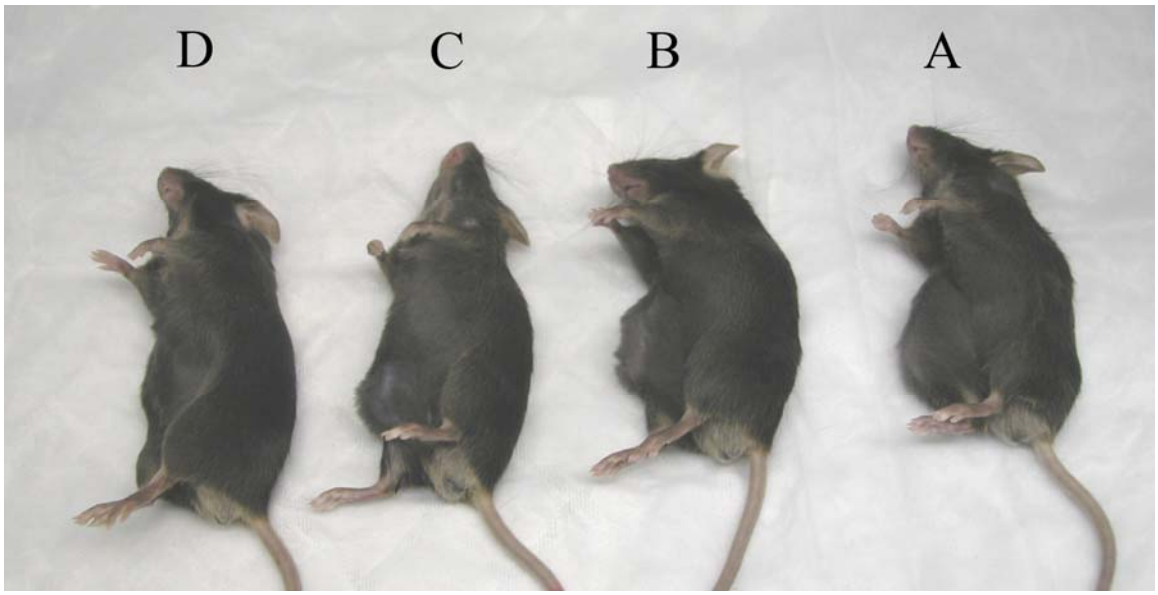


Figure 9.
Mice were injected with RM-1 murine prostate cancer cells. Treatment was started on day 5 by subcutaneous injection of the dendritic cells (opposite side of the tumor). Group 1 (control) mice were injected with the vehicle (HBSS), Group 2 – with DC tretaed by TNFα, Group 3 – with DC pulsed with RM-1 tumor lysates, Group 4 – treated with TNFα and BQ-788 (ET_B receptor inhibitor), and Group 5 – treated with TNFα and BQ-788 and pulsed with RM-1 tumor lysates. Tumor size was measured twice a week.

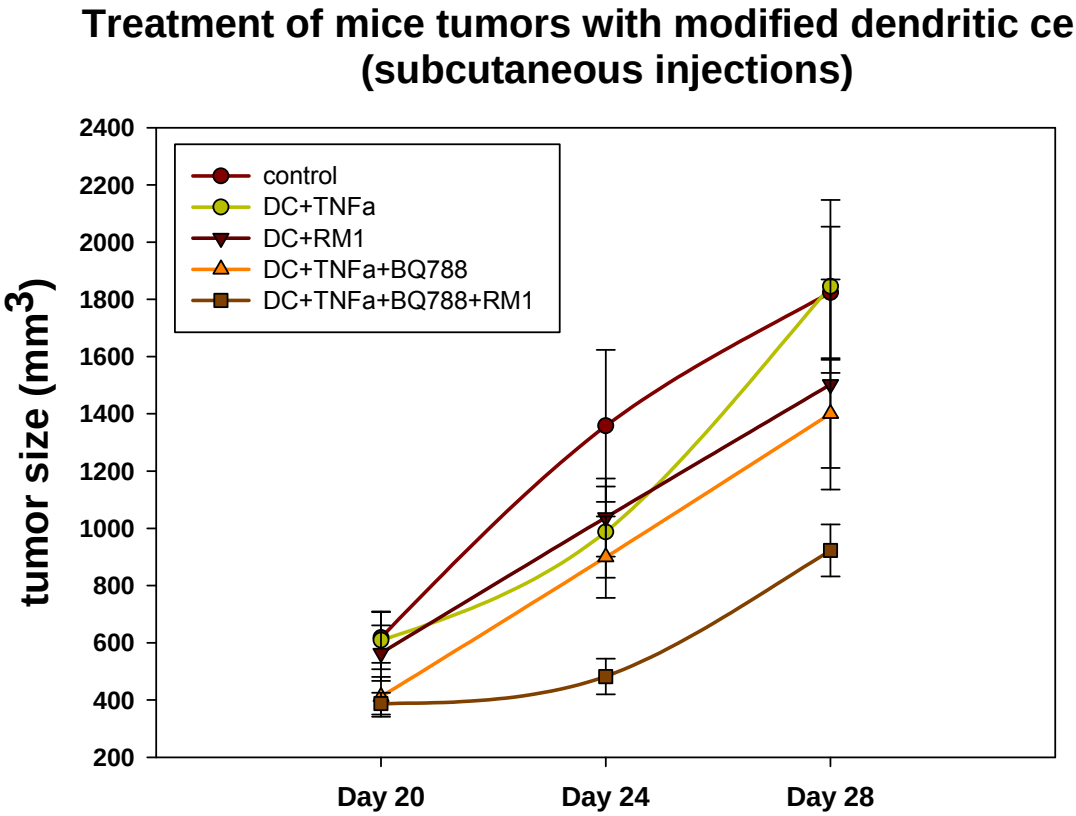


Figure 10.
Mice tumors at the end of experiment, representative samples. These mice were treated with modified and pulsed (with tumor lysates) dendritic cells, injected subcutaneously in the side opposite of tumor.
A – mouse treated with HBSS solution (control)
B – Mouse treated with DC + TNFa
C – Mouse treated with DC + RM-1 lysates
D – Mouse treated with DC + TNFa+ BQ-788 (endothelin B receptor inhibitor)
E – Mouse treated with DC + TNFa + BQ-788 + RM-1 lysates

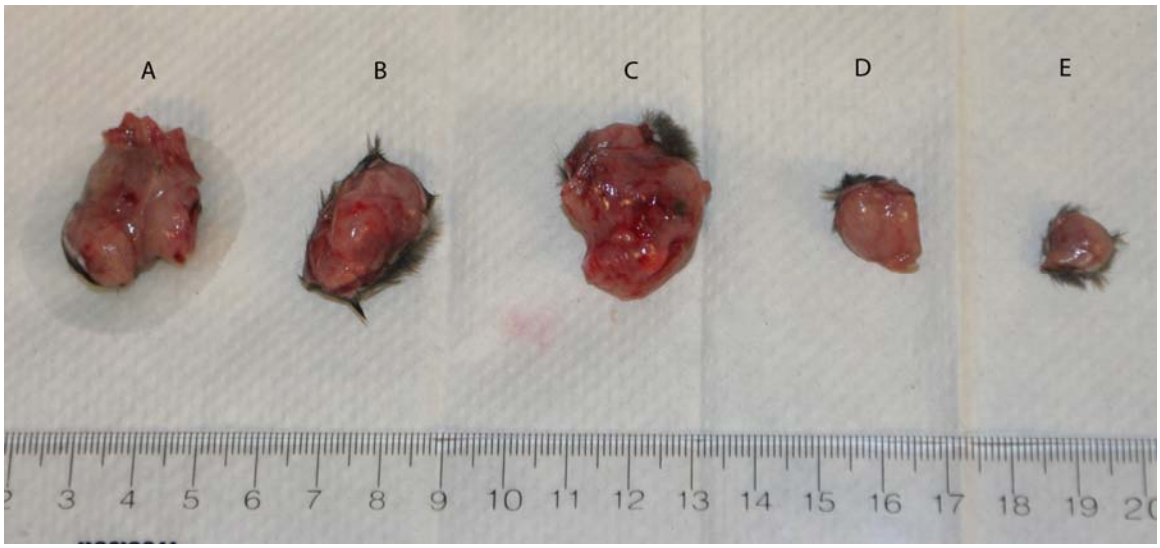
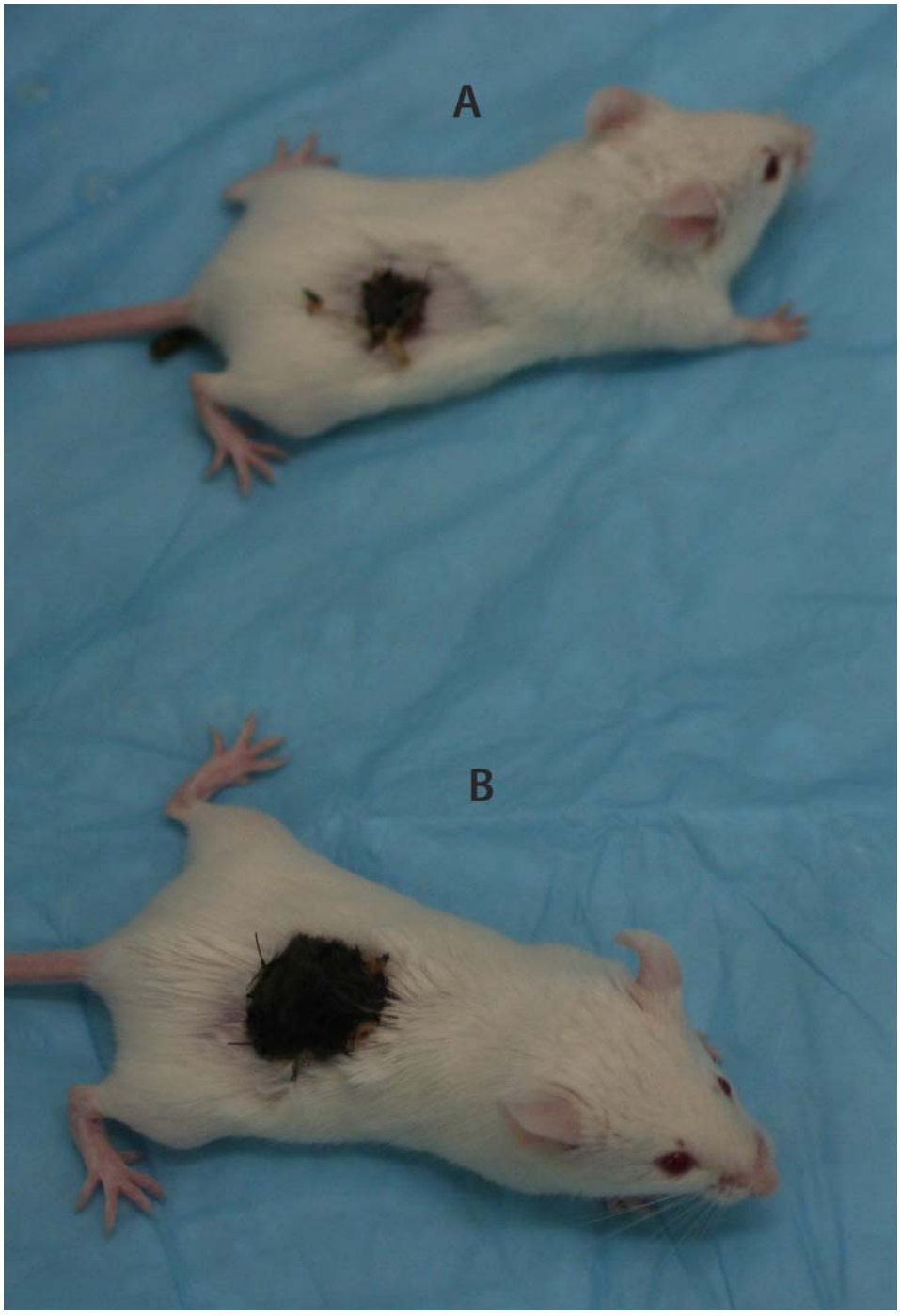


Figure 11.
Day 10 after skin transplantation.
A – control mouse, treated with intraperitoneal injection of vehicle (water), with necrosed scarring graft;
B – Mouse received daily intraperitoneal injections of ET_A receptor inhibitor BQ-123 (for 10 days). Skin graft is soft and viable.



Presented at the Annual Meeting of the AUA (May 2007, Anaheim, CA)

Prostate Cancer: Basic Research (III)

Moderated Poster
Sunday, May 20, 2007 3:30 PM - 5:30 PM

#531
USE OF MODIFIED DENDRITIC CELLS FOR THE TREATMENT OF PROSTATE CANCER IN MICE

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Introduction and Objective: Immunotherapy via dendritic cells (DC) has shown promise as a novel approach to hormone-refractory prostate cancer. DC are the major antigenpresenting cells and regulators of the immune response. We have previously demonstrated that prostate cancer induces DC apoptosis, which could be one mechanism of tumor escape from immune surveillance. We also demonstrated the presence of endothelin receptors on DC, and that blockade of endothelin B (ET_B) receptors decreases DC apoptosis, while it may enhance their antigen presenting ability. The purpose of the current study was to determine whether ET_B blockade also enhances DC antitumor activity in a murine prostate cancer model. Methods: Tumors were induced by subcutaneous injection of 25,000 RM-1 murine prostate cancer cells into groups (n=5) of the C57BL/6 mice. When tumors became palpable (day 6), treatment was initiated with injection of $1.5x10^6$ bone marrow derived DC into the tumor. DC were prepared as usual in the complete media in the presence of the GM-CSF and IL-4 for 7 days. Group 1 received Hank's solution (control); Group 2 - unmodified DC; Group 3 -DC treated with TNF-α during the last 48 hours; Group 4 - DC treated with TNF-α and ET_B receptor antagonist BQ-788 during the last 48 hours (our previous studies have shown the increased expression of endothelin receptors after the stimulation of DC with TNF-α). Two further injections were performed on days 9 and 12. Tumor size was assessed starting from day 14 until animal sacrifice. Results: By day 24, mean tumor size reached 1796 ± 166 mm^3 in the Group 1 (control), 1556 ± 186 mm^3 in the Group 2, $1508+166$ mm^3 in the group 3, and 397 ± 186 mm^3 in Group 4 ($P<0.001$ versus control). Difference in mean tumor size became significant starting from day 20. Conclusions: We hypothesize that ET_B receptor blockade protects DC from apoptosis and may increase their antigenpresenting capability. Our data suggest that ET_B receptor blockade also promotes DC antitumor activity *in vivo*. We plan to extend these studies into human clinical trials, using autologous dendritic cells.
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