Published in final edited form as:

Prostate. 2006 September 1; 66(12): 1275–1284.

MHC Class I and Class II Molecules Are Expressed in Both Human and Mouse Prostate Tumor Microenvironment

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Abstract

BACKGROUND—There has been a determined search for therapies specifically aimed at eradicating tumor cells while leaving normal host cells unaffected. This goal can potentially be accomplished by engaging tumor antigen-specific T-cell repertoire to attack the tumor. A prerequisite for a successful T-cell-mediated attack against tumors or pathogens is that the target tissues express major histocompatibility complex (MHC) molecules. Using newer anti-MHC class I and MHC class II antibody reagents, we re-examined the expression of MHC in both human and mouse prostate tumors and their microenvironments.

METHODS—Using immunocytochemistry, we examined the expression of MHC class I, class II, and CD3 molecules on cryopreserved human and mouse prostate tumor samples.

RESULTS—MHC class I molecules are expressed by the entire spectrum of different stages of both human and mouse prostate tumor cells. Additionally, cells of the hematopoietic lineage, dispersed in the tumor microenvironment, showed significant expression of MHC class II molecules. Human prostate tumors also show a significant infiltrate of CD3⁺T cells.

CONCLUSIONS—Expression of MHC class I and class II molecules within the prostate tumor microenvironment are consequential for T-cell-mediated immunotherapeutic approaches against prostate cancer.

Keywords

HLA; immunotherapy; T cells

INTRODUCTION

The traditional role of the cells of the immune system is to protect the individual against foreign pathogens by killing the pathogen and/or the cells infected with the pathogen. The specificity and destructive potential of lymphocytes is further apparent in autoimmune diseases in which T cells with the ability to recognize self proteins destroy the target tissue displaying the particular self antigens [1]. When a self or a non-self protein is exclusively expressed by tumor cells, the host T-cell repertoire directed to that antigen can be potentially activated to attack the tumor. We and others have shown that a repertoire of CD4 and CD8 T cells directed to prostate-specific proteins exists in humans [2–5]. To harness the potential of a T-cell repertoire specifically directed to attack prostate tumors, it is critical to understand the expression of

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molecular elements, within the tumor environment, that are essential to render the prostate tumors susceptible to an attack by the immune system.

Specific recognition of antigenic peptides bound within the antigen-binding groove of the major histocompatibility complex (MHC) class I and class II molecules is an obligatory first step to trigger a T-cell-mediated immune response. MHC (known as human leukocyte antigen [HLA] in humans) class I molecules display peptides derived from antigens expressed in intracellular compartments to cytotoxic CD8 T cells, and class II molecules exhibit peptides from extra-cellular sources to helper CD4 T cells [6,7]. Classical MHC class I molecules are ubiquitously expressed on all mammalian cells including cells of epithelial origin while MHC class II molecules are selectively expressed on antigen-presenting cells (APC) including dendritic cells (DC), macrophages, and B cells. Although APC constitutively express lower levels of MHC class II, activated APC express greatly enhanced levels of MHC class II molecules [8,9]. Furthermore, the expression of both the MHC class I and class II can be upregulated by cytokines such as γ -interferon (γ -IFN) and tumor necrosis factor (TNF) [10].

It has long been shown in several systems that cancer cells often downregulate expression of MHC class I molecules as a mechanism to evade recognition by T cells [11–13]. Analyses of human prostate tumors have shown either a complete lack or a reduced expression of MHC class I molecules [14–17]. In the same vein, expression of MHC class II molecules has also been shown to be lacking on human prostate cancer cells [14,16,18]. Since MHC molecules within the tumor are of key importance for designing any immunotherapeutic approach to prostate cancer, we have revisited the issue of MHC class I and class II expression within human prostate tumor microenvironment. Re-examination of this question seemed particularly essential due to a widespread use of an anti-human MHC class I antibody, W6/32, to detect MHC class I molecules in majority of these studies [14,15]. However, there has been a strong controversy over the ability of W6/32 antibody to recognize MHC-I in immunohistochemistry sections of human tissue [19,20]. Furthermore, some of the above-mentioned studies were done in sections of formalin-fixed, paraffin wax-embedded tissues [16,17]. It is known that the processing of tissues with conventionally used high concentrations of formalin as a fixative agent causes cross-linking in proteins sometimes leading to loss of antigenicity [21]. These controversies render the earlier results of a lack of or a reduced expression of HLA-I by human prostate cancer cells open to question.

In the present study, we have examined the expression of MHC class I and class II molecules by human prostate tumors in samples derived from 27 patients with prostate cancer as well as by prostate tumors derived from the TRAMP mice, a mouse model of prostate cancer [22]. We show that the prostate tumor microenvironment, in both the mouse and human systems, abundantly displays MHC class I on tumor epithelial cells and MHC class II on hematopoietic lineage cells scattered along with the prostate tumor cells. We also show a widespread infiltration of CD3⁺ cells within human prostate tumors. Our findings strongly suggest that the prostate tumor microenvironment is potentially permissive for a successful immunotherapeutic approach to prostate cancer than had been hitherto concluded.

MATERIALS AND METHODS

Patients and Human Tissues

The subject population consisted of 27 prostate cancer patients treated at Michael Reese Hospital and the University of Illinois between 1992 and 1995. Institutional Review Board approval was obtained for retrieval of prostate specimens in excess of that needed for pathologic diagnosis at the time of prostatectomy. Specimens were frozen in liquefied propane in the operating room and were subsequently stored in liquid nitrogen. Patients ranged in age from 45 to 75 years with a mean age of 67 ± 2 years. The patients were staged according to the

Whitmore/Jewitt Staging System with the following breakdown (n# in parenthesis): B₀ (1), B₁ (6), B₂ (7), C₁ (5), C₂ (7), D₂ (1). Gleason scores for the prostate tumors were assigned by a pathologist from the histology sections used for MHC analysis. Combined Gleason scores ranged between 6 and 10.

Mouse ProstateTissues

Prostate tumors were obtained from TRAMP mice which represents a spontaneous autochthonous transgenic model of prostate cancer [23]. TRAMP mice are transgenic mice in which a minimal probasin gene promoter was used to target expression of an oncoantigen, SV-40 large T-antigen, specifically to the epithelium of mouse prostate. The development and progression of prostate cancer in this model have been well characterized to closely resemble the human disease [22,23]. Six TRAMP mice were sacrificed between 18 and 32 weeks of age, prostate tumors were removed and frozen immediately in liquefied propane prior to storage in liquid nitrogen. Ventral and dorsolateral prostates from three transgene negative littermates (wild-type) were used for analysis of normal murine prostate. Tumor from one mouse was in an early stage of development, all others were fully developed tumors.

Immunocytochemistry

Prostatic tissue (human and mouse) was examined for expression of MHC-I, MHC-II, and CD3 molecules by an indirect immunocytochemical method, as previously described [24,25]. Briefly, frozen prostatic tissues were mounted on pre-cooled chucks (−20°C) in CM3050 cryostat (Leica Corp., Deerfield, IL). Sections (6 µm) were thaw-mounted on Superfrost Plus glass slides (Fisher Scientific, Itasca, IL). Mouse sections were freeze-substituted for 2 days in anhydrous acetone containing calcium chloride at −80°C. Both the human and mouse sections were then warmed to −20°C and transferred to 4°C where subsequent fixation and rinsing were performed. All sections were fixed in 2% paraformaldehyde (pH7.3) for 10 min, rinsed, incubated with 2% blocking serum (horse or goat), and incubated overnight at 4°C with primary antibody. For human tissues, mouse monoclonal anti-MHC-I (anti-HLA-A, -B, -C) antibody, G46-2.6, and anti-MHC-II (anti-HLA-DR, -DP, -DQ) antibody, TU39, anti-CD45 antibody, H130 (Pharmingen, now called Becton Dickinson Pharmingen [BD Pharmingen], San Diego, CA), and rabbit polyclonal anti-human CD3 (Dako Corp, Carpinteria, CA) at 1 µg/ml were used. The tissue treated with the primary antibody was reacted with biotinylated horse anti-mouse (rat absorbed) or biotinylated goat anti-rabbit secondary antibody (Vector Laboratories, Burlingame, CA) for 30 min at room temperature. For mouse tissues, biotinylated murine monoclonal anti-mouse MHC-I (anti-K^b/D^b) antibody, 28-8-6, anti-mouse MHC-II (anti-A^b) antibody, AF6-120.1 (BD Pharmingen), and rat anti-mouse CD45 antibody, 30-F11, were used at 2 µg/ml as the primary antibody. No secondary antibody was employed in the cases of anti-mouse MHC class I and class II antibodies and biotinylated rabbit anti-rat (mouse absorbed) (Vector Laboratories) antibody was used as the secondary antibody for mouse CD45 staining. Serial sections were stained with anti-MHC class II and anti-CD45 antibodies for both human and mouse prostate tumors in order to co-localize expression of MHC class II and CD45 on cells (Fig. 3). All antibodies had been described by the supplier to optimally detect MHC, CD45, or CD3 molecules, respectively, in sections of cryopreserved tissues. Furthermore, an optimal dose of antibodies used for staining was determined by preliminary dose response analyses. For both species, biotin was detected with an avidin-biotin peroxidase kit (ABC-Elite, Vector Laboratories) using diaminobenzidine tetrachloride as a chromogen. As a final step, the sections were dehydrated gradually with alcohol, cleared with xylene, and coverslipped with Permount (Fisher Scientific). Sections were counter-stained with Gill's #3 hematoxylin (1:4) as a blue nuclear counterstain. Substitution of normal isotype-matched mouse IgG or rat IgG (BD Pharmingen) for the primary antibody was used as a negative control for each adjacent section. Mouse thymus and mouse spleen sections processed in the same

manner were used as positive control tissues for antibodies directed to murine molecules (not shown).

RESULTS AND DISCUSSION

Human and Mouse ProstateTumor cells Abundantly Express MHC-I Molecules

Twenty-seven prostate cancer patients over a wide spectrum of grades and stages of prostate cancer, as depicted in Table IA, were used in the study. Prostate tumors from all 27 prostate cancer patients showed expression of HLA class I (HLA-I) molecules (Table IA, Fig. 1). Figure 1 shows staining of HLA-I molecules on human prostate cancer cells from three representative patients. HLA-I is expressed ubiquitously on most tumor epithelial cells (Panels A–C). Several cells dispersed in the vicinity of epithelial cells, typically expected to be of stromal or hematopoietic lineage, also display HLA-I molecules. However, expression of HLA-I on some stromal/hematopoietic cells, appears to be undetectable. The inset in each panel shows negative controls using isotype-matched normal IgG antibody as the primary antibody and it is clear that the normal antibody fails to show any non-specific staining. Grading for staining intensity for HLA-I revealed that most specimens demonstrated a high level of expression of HLA class I molecules (Table IA). As shown in Table IA, the mean levels of intensity for MHC-I staining did not correlate with Gleason scores of the patient.

Six prostate tumors from TRAMP mice were analyzed for this study. Tumor cells in all six mice expressed MHC-I molecules (Table IB). In addition, Table IB shows that the mean intensity of staining for MHC-I in different specimens of TRAMP tumors was moderate. Figure 2 (Panels A, B) shows staining from two representative mouse prostate tumors. Tumor epithelial cells from both mice show high level of expression of MHC-I molecules. Only one out of six TRAMP tumors was in early stage of tumor development, the remaining five being fully developed tumors. Thus correlation of MHC expression with the stage of tumor development was not possible. Prostate epithelial cells in the tissues from wild-type mice show a heterogeneous staining (Fig. 2, Panel C), with some cells expressing low levels of MHC-I, others displaying moderate levels of MHC-I and still others lacking expression of MHC-I molecules. The mean levels of intensity of MHC-I expression in the wild-type prostate were low (Table IB). The heightened expression of MHC class I on TRAMP tumor epithelial cells as compared to the wild-type prostate epithelial cells is expected to be due to inflammation accompanied by secretion of cytokines such as γ -IFN as a result of tumor development and tumor load in advanced stage TRAMP tumors.

Our results of an abundant expression of HLA class I molecules by the human prostate cancer cells are contrary to those of the previous reports of either decreased or a lack of expression of HLA class I by human prostate tumors [14–17]. We attribute the disparity in results in human prostate cancer being reported here and those reported earlier, to two main factors. One factor is the widespread use in the previous reports, of W6/32 antibody to detect human class I molecules. W6/32 antibody is a monoclonal antibody (mAb) which recognizes a non-polymorphic conformational epitope defined by residue 3 of β 2 microglobulin and a region, including residue 121, of the heavy chain of HLA-I molecules [26]. It has been shown that biotinylation of HLA-I molecules abrogates recognition by W6/32 antibody, although biotinylated HLA molecules are recognized quite well by an alternative antibody specific for another conformational epitope [19]. Furthermore, it has recently been shown by Blaschitz et al. [20] that W6/32 mAb poorly recognizes HLA-G2 molecules in immunocytochemical preparations of cell lines that expressed HLA-G2. HCA2, another mAb recognizing a non-polymorphic, but linear epitope of HLA class I molecules was, however, able to recognize HLA-G2 in analogous sections. Although there have been other reports that W6/32 mAb and HCA2 mAb can both recognize HLA-I in an equivalent manner on breast carcinoma tissue sections [27], these studies were not as adequately controlled as those of Blaschitz et al. [20].

The second factor causing discrepancy in our results and those reported in some earlier reports [16,17] probably involves preservation of antigenic epitopes during processing of tissue for immunocytochemistry. Thus, a recent report [17] demonstrated either a complete lack or a reduced expression of MHC class I molecules on human prostate tumors using HC-10 antibody. All tissue sections in this study were, however, derived from formalin-fixed paraffin wax-embedded specimens. It is well known that the conventional formalin treatment (4–10% formalin for several hours) of tissue could result in an extensive cross-linking of proteins leading to loss of antigenicity [21,28]. In addition, HC-10 antibody interestingly recognizes HLA-B and -C heavy chains and only a subset of HLA-A heavy chains [29]. Nevertheless, the controversy whether W6/32 antibody is able to recognize its conformational epitope on HLA class I molecules, as expressed on cells in histopathology tissue sections, combined with the use of less reliable methods of tissue processing, render the earlier results of a lack of or a reduced expression of HLA-I by human prostate cancer cells open to question.

We have now used a mAb which recognizes a non-polymorphic epitope of HLA class I and which has been shown by the suppliers (BD Pharmingen) to stain HLA class I molecules in cryopreserved immunocyto-chemical sections. Frozen sections of both the human and mouse prostate tumor tissues were fixed with a brief (10 min) and optimal treatment of 2% paraformaldehyde at the neutral pH, 7.3. Such fixation regimen used on frozen sections has been repeatedly shown to best preserve the antigenicity of proteins [26]. All 27 specimens of human prostate cancer express HLA-I in our analyses. These data are similar to the results of Sanda et al. [30] who showed normal expression of HLA-I on three of five human prostate cancer cell lines. Prior to our present report, TRAMP prostate tumors have not been examined for expression of MHC molecules. Our data of expression of MHC class I molecules on TRAMP tumor cells further support our observations in the human prostate cancer. We therefore believe that the results reported here reflect an accurate portrayal of the display of HLA class I molecules by human prostate cancer cells.

Microenvironment of Human and Mouse Prostate Tumors Displays MHC-II Molecules

Expression of HLA class II within three representative human prostate tumors is depicted in Panels D–F in Figure 1. Tumor epithelial cells mostly lack expression of HLA class II, thus confirming similar observations made earlier [14,16,18]. The present study, however, additionally shows that the cells, expected to be of stromal or hematopoietic origin, scattered within the microenvironment of prostate tumors express high levels of HLA class II molecules (Fig. 1 Panels D–F, Table I). As shown in Table I, there was little correlation of MHC class II expression with Gleason grades. Figure 2 shows expression of MHC class II on mouse prostate tumors. As is the case for human prostate cancer, the stromal/hematopoietic cells positioned in the microenvironment of mouse prostate tumors show strong expression of MHC class II (Fig. 2, Panels D, E). However, tumor epithelial cells lack expression of MHC class II.

To further explore whether MHC class II molecules are expressed on the stromal or the hematopoietic lineage cells, we stained serial sections of prostate tumors with anti-MHC class II and anti-CD45 antibodies. CD45 is known to be a marker on cells of the hematopoietic origin [31]. The data presented in Figure 3 indicate that all the cells expressing MHC class II also express CD45 molecules in both the human and mouse prostate tumor sections. Thus, expression of MHC class II molecules in the prostate tumor microenvironment is restricted to the hematopoietic lineage cells. The fact that the pattern of MHC class I and class II expression in the mouse prostate tumors is identical to that of the human prostate tumors is consistent with the fact that the TRAMP model of mouse prostate cancer has been shown to mimic its human counterpart [22,23]. The display of MHC class II in the wild-type prostate is the same as in the prostate tumors, that is, the epithelial cells lack expression of MHC-II, but cells of the hematopoietic lineage express MHC class II molecules (Table IB, Fig. 2, Panel F, CD45

staining data for wild-type mouse prostate not shown). The mean level of staining for the two wild-type prostate tissues was in the moderate range (Table IB).

Human Prostate Tumors Show Abundant Infiltration of CD3⁺ T Cells

Figure 4 shows a widespread presence of CD3⁺ lymphocytes in three representative human prostate tumors. As shown in Table I, tumors from all 27 patients showed a high infiltration of CD3⁺T cells and infiltration of T cells appeared to be equivalent in patients with different Gleason grades. These results are consistent with those reported by McClinton et al. and Bronte et al. [32,33] who also reported T-cell infiltration into human prostate tumors.

CONCLUSIONS

We conclude that in contrast to the previously held views, prostate tumor microenvironment, in both the mouse and human systems, plentifully displays MHC class I on tumor epithelial cells and MHC class II on cells of the hematopoietic lineage scattered along with the prostate tumor cells. We also show a widespread infiltration of CD3⁺ cells within human prostate tumors. These conclusions are significant since the expression of MHC class I and II molecules within the prostate tumor microenvironment is a finding vital for success of any immunotherapeutic approach involving specific T-cell-mediated attack against prostate tumors [2,34]. Expression of MHC class I on prostate tumors will enable the tumor cells to be perfect targets for CD8 T cells directed against prostate-specific proteins. In addition, expression of MHC class II on cells of the hematopoietic lineage, scattered within the prostate tumor microenvironment would insure activation and induction of helper function of prostate antigen-specific CD4 T cells. This is because of the fact that the conventional pathway of antigen presentation by MHC class II molecules samples antigens from extracellular sources, which are taken up by the cell, are processed and then presented by MHC class II molecules [7]. Any tumor or a tissue microenvironment that allows activation of both the CD4 and CD8 T cells is known to be more potent in permitting an effective T-cell attack against the target [2,34]. It is additionally encouraging that there is a large infiltrate of CD3 T cells within the prostate tumors since it reflects the ability of CD3 T cells to home to prostate tumors.

Despite the optimistic results of expression of MHC class I and class II molecules on prostate tumors, the tumors clearly not only escape an attack by the immune system but also have been shown to be a tolerogenic environment for T cells [33,35]. We hypothesize that as yet undefined molecular elements expressed within the tumor microenvironment lead to silencing of effector function of T cells and underlie the lack of impressive tumor regression in vaccinated cancer patients. Further studies are currently in progress in our lab as well as in other labs [33] to uncover what other molecules are either expressed or could be induced within the tumor microenvironment that might play a role in facilitating the expression of destructive potential of antigen-specific T cells. These studies will give us clues to mimic features of the site of an autoimmune attack within the tumor microenvironment with the aim of rendering the tumor as susceptible to T-cell attack as several autoimmune targets are [2,34]. It has been postulated that one mechanism causing the tolerogenicity of tumors would be the presence of immature APC including immature DC and macrophages [35]. It can be envisaged that a low level expression of MHC class II on immature APC will suffice to transmit the first signal (antigenic) for T-cell activation. However, a lack of expression of co-stimulatory molecules by immature APC could lead to the absence of the second signal required for T-cell activation and thus resulting in anergy of tumor antigen-specific T cells. Nevertheless, the data presented in Figure 3 of this manuscript indicate that the hematopoietic lineage cells within the tumor microenvironment are unlikely to be immature APC since they express rather high levels of MHC class II (see Fig. 3 A,C) and are likely to be activated APC. We speculate that despite the existence of activated potentially immunogenic APC within the prostate tumor

microenvironment, the latter still remains non-conductive to potent effector T-cell function [33]. Research is in progress in our lab and other labs, [see ref. 33] to identify comprehensive molecular mechanisms within the tumor microenvironment that may cause silencing of T-cell effector function. It is possible that once we overcome the properties of the tumor microenvironment that prohibit potent T-cell effector function, the concomitant expression of MHC class I and class II molecules within the tumor microenvironment will greatly facilitate novel immunotherapeutic approaches to attack prostate tumors.

Acknowledgements

This work was in part supported by American Cancer Society (Illinois Division) grant 97-45 (N.K.N) and National Institutes of Health grant DK-40890 (G.S.P).

Grant sponsor: American Cancer Society; Grant number: 97-45; Grant sponsor: National Institutes of Health; Grant number: DK-40890.

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Fig. 1.

MHC class I and class II molecules are expressed in human prostate tumor microenvironment. **A–C:** Human prostate tumor cells express HLA class I molecules: Immunocytochemical staining of cryopreserved sections of human prostate tumors derived from three patients, using mouse monoclonal anti-HLA class I (anti-HLA-A, -B, -C) antibody, G46-2.6, in an immunoperoxidase reaction, as described in Materials and Methods, is shown. A: Patient #7, Gleasonscore, 6.0. B: Patient #10, Gleasonscore, 7.0. C: Patient #30: Gleasonscore, 7.0. The inset shows staining with anisotype-matched normal IgG. Sections were counter-stained with hematoxylin. Majority of prostate tumor epithelial cells show expression of MHC class I molecules. Several stromal/hematopoietic cells also appear to express MHC class I molecules as well. e, epithelial cells, s, stromal cells. Magnification, 40×. **D–F:** HLA class II molecules are expressed by hematopoietic/stromal cells within the prostate tumor micro-environment: Immunocytochemical staining of cryopreserved sections of prostate tumors from three patients is presented in Panels D, E and F, respectively, as described above. Primary antibody used was mouse monoclonal anti-HLA class II (anti-HLA-DR, -DP, -DQ) antibody, TU39. HLA-II is mostly expressed by stromal/hematopoietic cells scattered in the vicinity of prostate tumor cells.

Fig. 2. MHC class I and class II molecules are expressed in the mouse prostate tumor (TRAMP) microenvironment. Immunocytochemical staining of cryopreserved sections of two TRAMP tumors, using murine monoclonal anti-mouse MHC-I (anti- K^b/D^b) antibody, 28-8-6 (**Panels A, B**), and anti-mouse MHC-II (anti- A^b) antibody, AF6-120.1 (**Panels D, E**), in an immunoperoxidase reaction, as described in Materials and Methods, is shown. **Panels (C)** and **(F)** are from frozen sections of prostate derived from TRAMP transgene negative littermates (wild-type) and stained for antibodies against MHC class I (Panel C) and MHC class II (Panel F) molecules, respectively. The insets are as described in Figure 1. Sections were counter-stained with hematoxylin. Majority of prostate tumor epithelial cells show high to strong level of expression of MHC class I molecules (Panels A, B). MHC class II molecules are expressed strongly, though mostly, on stromal/hematopoietic cells scattered within the prostate tumor microenvironment (Panels D, E). The sections from wild-type prostate show a low level expression of MHC class I (Panel C) and low to moderate level expression of MHC class II molecules (Panel F). e, epithelial cells; s, stromal cells. Magnification, 40 $\times$.

Fig. 3.

HLA class II molecules are expressed by CD45⁺ cells of the hematopoietic lineage present in the human and mouse prostate tumor microenvironment: Immunocytochemical staining of cryopreserved sections of prostate tumors from one prostate cancer patient (**Panels A and B**) and one TRAMP mouse (**Panels C and D**) is presented. Primary antibodies used were: mouse monoclonal anti-HLA class II antibody, TU39 (Panel A), anti-human CD45 antibody (Panel B), anti-mouse MHC class II antibody (Panel C), and anti-mouse CD45 antibody (Panel D). MHC-II is mostly expressed by CD45⁺ hematopoietic cells scattered in the vicinity of prostate tumor cells. The staining was performed and is depicted as described in Figure 1 and in Materials and Methods. Arrows in panels A and B; and C and D correspond to cells observed in the serial sections respectively. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Fig. 4.

Human prostate tumors show abundant infiltration of CD3+ T cells: Immunocytochemical staining of cryopreserved sections of prostate tumors from three patients is presented in Panels A–C as described in Figure 1. The primary antibody used was rabbit polyclonal anti-human CD3. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE I
TABLE IA. Levels of MHC Class I and Class II Expression and T Lymphocytic Infiltration Within the Human Prostate Tumor Milieu
TABLE IB. Levels of MHC Class I and Class II Expression Within the Mouse Prostate Tumor Milieu

Gleason grades	n ^a	Stage	MHC class I	MHC class II	CD3 ⁺ T-cell infiltration
6	6	B ₁ -C ₂	2.8 ^b	2.0	3.0
7	16	B ₁ -C ₂	3.4	2.0	2.6
8-10	5	B ₀ -D ₂	2.5	2.9	2.9
Tissue	n	MHC class I	MHC class II		
TRAMP tumors	6	2.0	2.0		
Wild-type prostate	2	1.0	2.0		

^a n is the number of specimens included in each group. All specimens showed expression of MHC I, MHC II, and CD3 molecules.
^b Numbers (in columns MHC class I, MHC class II, and CD3 T-cell infiltration) represent mean intensity level of staining for specimens included in each group after taking into account proportion of cells staining in a given field. Thus, for the case of MHC class II, the numbers represent staining in the context of the entire field of view that includes tumor cells which are negative for staining with anti-MHC class II antibodies. Each specimen was scored for levels of staining in arbitrary numbers as follows: 1, low; 2, moderate; 3, high; 4, strong.