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TITLE: Exploiting the Innate Antitumor Activity of Human Gamma-Delta T-Cells for the Treatment of Prostate Cancer

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**Exploiting the Innate Antitumor Activity of Human Gamma-Delta T-Cells for the Treatment of Prostate Cancer**

**Purpose and scope:**

The aims of this project are, 1) to more precisely characterize the extent and breadth of the antitumor cytotoxicity mediated by apoptosis-resistant human γδ-T cells against human prostate cancer cells; 2) to define the general mechanisms involved in the recognition and lysis of sensitive prostate cancer cells by apoptosis-resistant γδ-T cells; and 3) to determine the extent to which apoptosis-resistant γδ-T cells can regulate the growth and metastasis of prostate cancer cells in vivo. **Key findings to date:** 1) γδ-T cells derived from a variety of donors consistently displayed lytic activity against prostate cancer cell lines DU-145 and PC-3, but not LNCaP. 2) Monoclonal antibodies (mAb) against either the Vγ9 or Vδ2 TCR chains as well as mAb against intercellular adhesion molecules-1 (ICAM-1) or CD18 (β subunit of the β2 integrins) blocked γδ-T cell-mediated killing of prostate cancer cells; 3) γδ-T cells lyse prostate cancer cell lines largely through the perforin/granzyme pathway. 4) Using the TRAMP transgenic mouse model of prostate cancer, we have shown that the absence of γδ-T cells is indeed permissive for the development of tumors.
INTRODUCTION

We initially identified and characterized a CD2-mediated, interleukin (IL)-12–dependent signaling pathway which inhibits apoptosis in mitogen-stimulated human γδ-T cells. We have since exploited this pathway to develop the methodologies allowing the large-scale ex vivo expansion of viable apoptosis-resistant γδ-T cells – an undertaking until now, not possible. Importantly, we have shown that apoptosis-resistant human γδ-T cells retain significant innate, major histocompatibility complex (MHC)-unrestricted cytotoxicity against a wide variety of human-derived tumor cell lines, including human prostate cancer cell lines. Our efforts related to this proposal have remained focused upon testing the hypothesis that γδ-T cells – by virtue of their innate ability to recognize and kill epithelial-derived malignancies – play an important role in regulating the initial growth or spread of prostate cancer in vivo. The specific aims of this project are, 1) to more precisely characterize the extent and breadth of the antitumor cytotoxicity mediated by apoptosis-resistant human γδ-T cells against human prostate cancer cells; 2) to define the general mechanisms involved in the recognition and lysis of sensitive prostate cancer cells by apoptosis-resistant γδ-T cells; and 3) to determine the extent to which apoptosis-resistant γδ-T cells can regulate the growth and metastasis of prostate cancer cells in vivo.

BODY

In the initial period of this grant for which this report is generated (April 1, 2003 to March 1, 2004) our accomplishments are presented in relation to the following tasks as outlined in the approved Statement of Work.

Task 1. To characterize the extent and breadth of the antitumor cytotoxicity mediated by apoptosis-resistant human γδ-T cells against human prostate cancer cells

We have re-prioritized the order in which we are approaching out tasks. All patient-related studies have been moved back 12 months, as such, no results are presented here.

Task 2. To define the general mechanisms involved in the recognition and lysis of sensitive prostate cancer cells by apoptosis-resistant γδ-T cells.

Initial Objectives and Rationale. Here, we proposed to determine the extent to which various known molecular receptors are used by apoptosis-resistant γδ-T cells to recognize sensitive prostate cancer cells. We also proposed to determine which molecular ligands present on sensitive prostate cancer cells (such as MICA or other MHC class-I–like molecules) are recognized by apoptosis-resistant γδ-T cells. In addition, we proposed to define the extent to which known adhesion molecules are involved in the cellular interactions between apoptosis-resistant γδ-T cells and sensitive prostate cancer cells. Finally, we proposed to determine the extent to which granule exocytosis or Fas/FasL–mediated mechanisms are utilized by apoptosis-resistant γδ-T cells in the killing of sensitive prostate cancer cells.

Findings. The key findings related to this task are presented in the attached draft of the manuscript entitled, "Ex vivo expanded human Vγ9Vδ2+ γδ-T cells mediate innate antitumor activity against human prostate cancer cells in vitro". This manuscript is to be submitted shortly. In summary, (taken from the manuscript) we have found:

- γδ-T cells derived from a variety of donors consistently displayed lytic activity against prostate cancer cell lines DU-145 and PC-3, but not LNCaP.
- Monoclonal antibodies (mAb) against either the Vγ9 or Vδ2 TCR chains as well as mAb against intercellular adhesion molecules-1 (ICAM-1) or CD18 (β subunit of the β2 integrins) blocked γδ-T cell-mediated killing of prostate cancer cells.
- γδ-T cells lysed prostate cancer cell lines largely through the perforin/granzyme pathway.
From these findings we can conclude that ex-vivo expanded human Vγ9Vδ2+ γδ-T cells are able to innately recognize and kill certain human prostate tumor cell lines in vitro. Recognition and killing of prostate cancer cells occurs in a γδ-TCR—dependent manner and also appears to involve adhesion occurring through ICAM-1 and CD18. The cytolytic process involves primarily the perforin/granzyme mediated pathway of granule exocytosis.

**Task 3. To determine the extent to which apoptosis-resistant γδ-T cells can regulate the growth and metastasis of prostate cancer cells in vivo**

*Initial Objectives and Rationale.* Our initial intent of these studies was to use a transgenic mouse model of prostate cancer (TRAMP) to define the role of γδ-T cells in controlling the development and growth of prostate cancer. This was to be accomplished by classical genetic means where TRAMP mice will be bred with γδ-T cell knockout mice (TCRδ-/-) — animals which we then predict will develop tumors more rapidly and extensively. TRAMP-derived prostate cancer cell lines will also be used to establish tumors in syngeneic TCRδ-/- mice. We then propose to rescue these animals by the adoptive transfer of syngeneic apoptosis-resistant murine γδ-T cells (which we can also now readily generate and isolate).

*Findings.* Using the TRAMP transgenic mouse model of prostate cancer, we have shown that the absence of γδ-T cells is indeed permissive for the development and growth of prostate tumors as demonstrated in *Figure 1* (unpublished data). TRAMP mice develop prostate cancer in a predictable manner. By back-crossing TRAMP mice with mice lacking γδ-T cells (TCRδ-/-), we observe that TRAMP × TCRδ-/- mice (*Figure 1C*) develop more aggressive prostate cancers at a far younger age in comparison to control TRAMP animals (*Figure 1B*).

*Figure 1. Absence of γδ-T cells is permissive for the development of cancer.* As described and referenced in the text, TRAMP mice are transgenic for a construct consisting of the minimal rat probasin promoter which drives expression of the SV40 early genes (T and 1; Tag) in a prostate tissue-specific manner. As a consequence, TRAMP mice spontaneously develop prostate adenocarcinomas in a predictable manner. In order to determine if the absence of γδ-T cells is permissive for the development of adenocarcinoma, TRAMP mice were first backcrossed with commercially available TCRδ-chain knockout mice (TCRδ-/- mice, Jackson Labs, Bar Harbor, ME) which fail to develop γδ-T cells. *A* Normal genitourinary (GU) tract of a male wild-type (C57BL/6) mouse surgically removed at 7 months of age. *B* GU tract of a male TRAMP mouse surgically removed at 7 months of age. Tumor infiltration of GU structures (including bladder, epididymis and prostate) were observed grossly and microscopically (not shown) in a manner consistent with published data. *C* GU tract of a male TRAMP × TCRδ-/- animal removed at 5 months of age. Gross effacement of all GU structures was observed. Early sacrifice of TRAMP × TCRδ-/- animals was performed in accordance with animal welfare guidelines. Not shown, TRAMP × TCRδ-/- animals (of γδ-T cell-deficient) had GU tracts similar to age-matched TRAMP animals.
Findings. We have also been able to transfer TRAMP cell lines into TCRδ−/−, TCRβ−/− and control C57BL/6 mice. We are now treating these animals with mouse γδ-T cells derived from wild-type C57BL/6 mice. We have no definitive results to report at this early time.

KEY RESEARCH ACCOMPLISHMENTS

- We can now conclude that ex-vivo expanded human Vγ9Vδ2+ γδ-T cells are able to innately recognize and kill certain human prostate tumor cell lines in vitro. Recognition and killing of prostate cancer cells occurs in a γδ-TCR—dependent manner and also appears to involve adhesion occurring through ICAM-1 and CD18. The cytolytic process involves primarily the perforin/granzyme mediated pathway of granule exocytosis.
- We have derived key animal data which support our view that the absence of γδ-T cells is indeed permissive for the development of prostate cancer using the TRAMP mouse model. These are key proof-of-principal studies that support our overall hypothesis.

REPORTABLE OUTCOMES

- none to date

CONCLUSIONS

- As noted above, the findings that we report here are quite preliminary, but support our overall hypothesis that "By virtue of their ability to innately recognize and kill epithelial-derived malignancies, human γδ-T cells play an important role in regulating the initial growth or spread of prostate cancer in vivo".

REFERENCES

- none

APPENDIX

- draft of manuscript entitled, "Ex vivo expanded human Vγ9Vδ2+ γδ-T cells mediate innate antitumor activity against human prostate cancer cells in vitro", to be submitted shortly.
Ex vivo expanded human Vγ9Vδ2+ γδ-T cells mediate innate antitumor activity against human prostate cancer cells in vitro

ABSTRACT

Purpose:

Unlike αβ-T cells, γδ-T cells recognize tumor cells in a major histocompatibility complex (MHC)-independent manner which requires no processing or presentation of tumor-specific antigens. Consequently, γδ-T cells can recognize and lyse tumor cells almost immediately upon encounter – consistent with their role as a component of the innate immune system. Strategies designed to exploit this innate antitumor activity of human γδ-T cells for the treatment of cancers, including prostate cancer, are appealing for this reason. We have previously identified a CD2-mediated, IL-12-dependent signaling pathway which inhibits activation-induced cell death (AICD) in mitogen-stimulated human γδ-T cells which in turn permits the large-scale expansion of apoptosis-resistant γδ-T cells, most of which express the Vδ2 T cell receptor (TCR) chain, in combination with the Vγ9 TCR chain. While it is well documented that γδ-T cells which express the Vδ1 TCR chain display broad cytolytic activity against a variety of epithelial-derived human tumors, less is known regarding the antitumor activity of Vγ9Vδ2+ γδ-T cells, particularly against epithelial-derived malignancies. Here we describe the innate antitumor activity of ex vivo expanded human Vγ9Vδ2+ γδ-T cells against prostate cancer cells.

Materials and Methods:

Apoptosis resistant human γδ-T cells were first expanded in vitro from cultured human peripheral blood mononuclear cells (PBMC) then enriched to high purity utilizing standard immunomagnetic separation technology. In vitro cytotoxicity of expanded apoptosis resistant γδ-T cells was measured against human prostate cancer-derived cell lines and was determined utilizing standard four hour ⁵¹Cr-release assay.

Results:

γδ-T cells derived from a variety of donors consistently displayed lytic activity against prostate cancer cell lines DU-145 and PC-3, but not LNCaP. Monoclonal antibodies (mAb) against either the Vγ9 or Vδ2 TCR chains as well as mAb against intercellular adhesion molecules-1 (ICAM-1) or CD18 (β subunit of the β₂ integrins) blocked γδ-T cell-mediated killing of prostate cancer cells. γδ-T cells lysed prostate cancer cell lines largely through the perforin/granzyme pathway.

Conclusion:

Ex-vivo expanded human Vγ9Vδ2+ γδ-T cells are able to innately recognize and kill certain human prostate tumor cell lines in vitro. Recognition and killing of prostate
cancer cells occurs in a γδ-TCR–dependent manner and also appears to involve adhesion occurring through ICAM-1 and CD18. As apoptosis-resistant human Vγ9Vδ2+ γδ-T cells can readily be expanded to large numbers in our laboratory, these findings must be considered in the context of developing new adoptive cellular therapy approaches for the treatment of prostate cancer.

Key Words:

γδ-T cell; cytotoxicity; prostate cancer; innate immunity; adoptive immunotherapy.

INTRODUCTION

Adenocarcinoma of the prostate remains one of the leading causes of cancer-related deaths among American men. Although current standard therapies for early-stage prostate cancer – including surgery, radiotherapy or hormonal blockade – are usually very effective at achieving initial disease control, prostate cancer often recurs. Moreover, salvage chemotherapy for recurrent prostate cancer – or chemotherapy for prostate cancer presenting initially as wide-spread metastatic disease – is often associated with very poor responses. Clearly, new forms of therapy for recurrent or metastatic prostate cancer are needed.

The view that the immune system itself might be exploited for the treatment of prostate cancer is not new. Interestingly, to date the overwhelming majority of reports in this regard have focused primarily upon augmenting the adaptive immune response to prostate cancer-specific antigens. This includes a number of important studies designed to induce tumor-specific cytotoxic CD8+ αβ-T lymphocytes (CTL) utilizing prostate cancer-specific peptide antigens, as well as other studies designed to develop tumor-specific immune responses employing dendritic cell-based vaccination strategies (1-15). However, these and similar approaches which rely primarily upon adaptive immunity suffer from several potential shortcomings.

First, these strategies presuppose that an antigen selected as a target for immune-based therapy is indeed, tumor-specific – that is, the antigen is expressed only in tumor cells, but not in normal tissues. This problem is illustrated when one considers studies designed to target prostate specific antigen (PSA) as a tumor-specific immunogen: While PSA is expressed by the vast majority of prostate cancer cells, it is also expressed by normal prostate tissues. Moreover, PSA might not be expressed at all by some poorly differentiated adenocarcinomas.

Secondly, it is well established that prostate cancer cells can either downregulate the expression of MHC class-I or class-II molecules, or suffer from defects in assembly and expression of MHC class-I (10, 16, 17). Thus, tumor cells expressing little or no MHC molecules might selectively escape recognition by MHC-restricted CD8+ CTL or CD4+ T helper cells. These latter findings emphasize the importance of developing immunotherapy strategies that do not require or even utilize classical MHC-restricted antigen processing and presentation.
Unlike αβ-T cells, γδ-T cells recognize tumor cells in an MHC-independent manner, requiring no processing or presentation of tumor-specific antigens. Instead, γδ-T cells recognize a variety of MHC class-I-like antigens which are commonly displayed by cells having undergone malignant transformation, particularly cells of epithelial origin (18-20). Indeed, γδ-T cells may provide an alternative or complementary means of recognizing and killing tumor cells which have escaped adaptive immune responses.

In peripheral blood, γδ-T cells represent only a minor fraction of total T cells, usually only 1 to 5 percent (21). The vast majority of peripheral blood γδ-T cells express the Vγ9 TCR chain usually in combination with the Vδ2 TCR chain (Vγ9Vδ2 γδ-T cells, also termed Vγ2Vδ2 in an alternate nomenclature). It is thought that Vγ9Vδ2 γδ-T cells provide some degree of immunosurveillance against intracellular pathogens and certain hematological malignancies (22-24). In contrast, γδ-T cells found in epithelial tissues (such as the intestine, skin, tongue, esophagus, trachea, lungs and genital tract) usually express the Vδ1 TCR chain in combination with a variety of Vγ chains (25). Vδ1 γδ-T cells can be found in association with or even infiltrating some solid tumors and often display MHC-unrestricted lytic activity against a variety of cancer cells – particularly those of epithelial origin (25).

We have previously identified a CD2-mediated, IL-12-dependent signaling pathway which inhibits apoptosis in mitogen-stimulated human γδ-T cells derived from peripheral blood (26). This in turn allows for the large-scale expansion of apoptosis-resistant γδ-T cells, most of which express the Vγ9Vδ2 TCR.

Although it is been reported that activated Vγ9Vδ2 γδ-T cells are capable of in vitro killing of human lymphoma cell lines, little is known regarding the innate antitumor activity of Vγ9Vδ2 γδ-T cells as measured against human epithelial-derived cancer cell lines. Here we describe the innate antitumor activity of ex vivo expanded human Vγ9Vδ2 γδ-T cells against human prostate cancer cell lines and discuss this finding in the context of developing new strategies for the adoptive cellular therapy of prostate cancer.

**MATERIALS AND METHODS**

**Tumor cell lines and antibodies.**

Human prostate cell lines including DU145, LNCaP and PC-3 were obtained from American Type Culture Collection (ATCC, Manassas, VA) and cultured in the appropriate medium as recommended by ATCC.

**Preparation of γδ-T and αβ-T cells from PBMC cultures**

Ex-vivo expanded apoptosis resistant γδ-T cells were prepared as we have previously described (26). Briefly, PBMC were isolated by Ficoll gradient centrifugation of whole
blood obtained from healthy human volunteers. Cultures were initiated at a cell density of $1 \times 10^6$ cells/mL in RPMI-1640 with 10% fetal bovine serum (HyClone, Logan, UT), 2 mmol/L L-glutamine, 100 U/mL penicillin, 100 U/mL streptomycin, and 50 μmol/L 2-ME. On the day of culture initiation (day 0), human recombinant interferon (rIFN)-γ (1000 U/mL; Boehringer Mannheim, Indianapolis, IN); human rIL-12 (10 U/mL; R&D Systems, Minneapolis, MN), and mouse anti-human CD2 mAb clone S5.2 (1-10 μg/mL, mouse IgG2a; Becton Dickinson, San Jose, CA) were added. Twenty-four hours later (day 1), cultures were stimulated with 10 ng/mL anti-CD3 mAb OKT3 (mouse IgG2a; OrthoBiotec, Raritan, NJ) and 300 U/mL human rIL-2 (Boehringer Mannheim). Fresh medium with 10 U/mL IL-2 was added every 7 days. The expanded cultures were subjected to γδ-T cell positive selection for γδ-T cells, and γδ-T cell depletion for αβ-T cell selection by immunomagnetic column separation on day 13 or 14. Cells were first stained with an anti-γδ-TCR mAb conjugated to magnetic beads (Miltenyi Biotec, Auburn, CA) then passed through an immunomagnetic cell sorter (AutoMACS, Miltenyi Biotec) using the manufacturer’s instructions. Isolated cells were washed with PBS and cultured overnight in complete RPMI medium with 100U/ml of IL-2. Purity of isolated γδ-T cells and αβ-T cells was assessed by flow cytometry and routinely found to be > 95 percent with > 90 percent viability.

**Chromium $^{51}$Cr-release cytotoxicity assay**

Target cells were labeled with 100 μCi Na$_2$ $^{51}$CrO$_4$ (Amersham Pharmacia Biotech, Piscataway, NJ) overnight at 37°C, after which cells were washed, trypsinized, and suspended in RPMI containing 10% FBS. Cells were then plated (2 $\times$ $10^3$/well) in 96-well V-bottom microtiter trays. Purified αβ- or γδ-T cells in varying numbers were added to target cells in a final volume of 150 μL. Trays were briefly centrifuged and then incubated for 4 hours at 37°C, after which 50 μL supernatant was removed to determine $^{51}$Cr release in cpm. Percentage of specific target cell lysis was calculated as $\frac{(\text{experimental release}-\text{spontaneous release})}{(\text{maximum release}-\text{spontaneous release})} \times 100$. Maximum and spontaneous releases were respectively determined by adding either 0.1% Triton X-100 or culture medium alone to labeled target cells in the absence of effector cells. Data are presented as the mean (± SD) of triplicate samples.

**Flow cytometry analysis of cells**

Flow cytometry was performed as we previously described (26). Briefly, cells were stained with directly fluorescence-conjugated mAbs recognizing TCR-δ1, TCR-δ2 and CD3 separately. Directly fluorescence-conjugated isotype-matched irrelevant antibodies served as controls. Analyses were performed using a FACSCalibur flow cytometer (Becton Dickinson). Propidium iodide (PI) uptake was used to exclude non-viable cells during analysis. Data analysis was performed using CellQuest software (Becton Dickinson).
RESULTS

Ex-vivo expanded apoptosis resistant γδ-T cells express primarily the Vγ9Vδ2 TCR

To determine the usage of TCR on expanded apoptosis resistant γδ-T cells, total γδ-T cells were first isolated from PBMC cultures by immunomagnetic column separation as described above. Purified cells were cultured for 24 hours prior to analysis to allow mAb used for sorting to dissociate. Anti-Vδ1 and Vδ2 mAb conjugated with FITC and PE respectively as well as anti-CD3 mAb with APC were incubated with the cells. After being washed, the cells were analyzed by FACS. PI negative events were gated for analysis to exclude non-viable cells. The results are shown in Figure 1 where 96% of gated cells display CD3 and Vδ2 on their surface, while essentially no Vδ1 γδ-T cells were detected in the expanded cultures.

Vγ9Vδ2 γδ-T cells exhibit cytotoxicity activity against some prostate tumor cells

It is established that activated Vγ9Vδ2 γδ-T cells exhibit cytotoxicity activity to lymphoma cell lines. However, the ability of these cells to recognize and lyse malignant cell lines of epithelial origin has not been well described. Here, we examined cytolytic activity of apoptosis resistant Vγ9Vδ2 γδ-T cells against human prostate tumor cell lines DU145, PC-3 and LNCaP. PBMC were isolated from healthy donors then cultured as described above. After 14 days, apoptosis-resistant γδ-T cells were sorted to high purity utilizing the Miltenyi AutoMACS immunomagnetic cell separator. Human prostate cancer cell lines DU145, PC-3 and LNCaP were first labeled with 51Cr, then incubated with γδ-T cells or control αβ-T cells. After a 4 hour incubation at the indicated E:T ratios, supernatants were removed to determine 51Cr release in CPM. Data are expressed as the mean percent specific target lysis (± SD) of triplicate determinations. These studies are representative of experiments performed 3 other times. These findings indicate that apoptosis resistant γδ-T cells can specifically lyse human prostate cancer cell lines DU145 and PC-3 LNCaP (Figure 2).

Involvement of TCR in the lysis of sensitive tumor cells by Vγ9Vδ2 γδ-T cells

Vδ1 γδ-T cells are able to kill a wide variety of epithelial derived tumor cells in vitro, as has been reported. Above, we have demonstrated that Vγ9Vδ2 γδ-T cells are also able to recognize some epithelial tumor cells such as DU145 and PC-3. To determine whether the γδTCR itself is involved in the recognition and killing of tumor cells, blocking antibodies to either TCR chain Vγ9 or Vδ2 were added to effector γδ-T cells prior to coculture with tumor target cells (Figure 3). Both antibodies inhibited specific lysis, suggesting that TCR participates in the γδ-T cell-mediated lysis of tumor cells. The function of TCR in antitumor was also verified in cytotoxicity assays by the addition of pan-γδTCR mAbs which recognize all γδ-T cells, regardless of TCR chain expression.

Involvement of additional cell surface structures in the recognition and lysis of sensitive tumor cells by Vγ9Vδ2 γδ-T cells

Similar studies were performed utilizing a number of mAbs to known surface structures likely to be involved in the interaction between cytotoxic γδ-T cells and sensitive prostate cancer cell line PC-3. In a number of studies performed, only mAb to CD18 and CD54...
were able to consistently inhibit \( \gamma^\delta \)-T cell-mediated killing of PC-3 cells, suggesting that interactions between CD18 and CD54/ICAM-1 may be involved in the recognition and killing of prostate cancer cells by \( \gamma^\delta \)-T cells (Figure 3).

**\( \gamma^\delta \)-T cell-mediated cytolysis of tumor cells involves perforin/granzyme pathway**

Two independent pathways, granule exocytosis and Fas-induced apoptosis have been reported in cytotoxic T lymphocytes (CTL) mediated cytotoxicity.\(^{13}\) As \( \gamma^\delta \)-T cells constitutively express perforin and granzymes (serine esterase) we examined whether perforin/granzyme exocytosis as a mechanism involved in lysis of tumor cells. As granule exocytosis is \( \text{Ca}^{2+} \)-dependent, studies cytolysis assays were performed in the presence of \( \text{Ca}^{2+} \)-chelators. As shown in Figure 4, lysis of DU145 by \( \gamma^\delta \)-T cells was blocked by the addition of EGTA-Mg\(^{2+} \) (depletion of \( \text{Ca}^{2+} \)), but is partially restored by \( \text{Ca}^{2+} \) replacement – suggesting that \( \gamma^\delta \)-T cell-mediated cytolysis of prostate cancer cells depends involves the perforin/granzyme pathway.

**Discussion**

It has been well established that human \( V\delta 1 \) \( \gamma^\delta \)-T cells can recognize and kill sensitive human tumor cells through the recognition of MHC class I-related molecules MICA and MICB which are expressed on malignantly transformed cells (27). This interaction appears to occur through NKG2d, a receptor expressed by subsets of cytolytic T cells and natural killer (NK) cells. MICA and/or MICB also appear to interact directly with the \( V\delta 1 \) TCR (18-20, 28).

In this current study, we report that ex vivo expanded \( \gamma^\delta \)-T cells recognize and kill epithelial-derived tumor cells in a \( \gamma^\delta \)-TCR-dependent manner. However, it is still unclear whether MICA and/or MICB are directly involved in this process. Indeed, we observed no difference (data not shown) in MICA and MICB expression on cells which were sensitive to \( \gamma^\delta \)-T cell-mediated killing (DU145 and PC-3) and cells that were resistant to killing (LnCap). We did observe however that expression of CD54/ICAM-1 was impaired in the cell line LNCaP which was not killed by \( \gamma^\delta \)-T cells (data not shown), this suggesting that ICAM-1 interactions with ligands expressed on \( \gamma^\delta \)-T cells may be important in the process of recognition and killing of sensitive tumor target cells. This is supported by our findings that blocking antibodies to either CD18 or CD54/ICAM-1 inhibited \( \gamma^\delta \)-T cell-mediated tumor cell lysis. Studies to more directly address this issue are currently underway and will be reported separately.

Though by no means exhaustive, our finding that ex vivo expanded, apoptosis-resistant human \( \gamma^\delta \)-T cells can recognize and kill human prostate cancer cells is important for a number of reasons. First, this finding is consistent with the emerging model that human \( \gamma^\delta \)-T cells can indeed recognize and lyse a wide variety of human epithelial-derived tumors. Importantly, here we show that \( \gamma^\delta \)-T cells are quite capable of killing epithelial-derived tumor cells, a function that is often more associated with \( V\delta 1 \) \( \gamma^\delta \)-T cells. Second, these findings in addition to our previous studies establish that we can
indeed expand large numbers of prostate cancer-reactive Vγ9Vδ2 γδ-T cells, a finding that has never been reported. These two points taken together provide a rationale – which is both biologically sound and practical – for proposing further studies to determine how human γδ-T cells might be exploited for the adoptive immunotherapy of prostate cancer.

These types of studies eventually may allow us to address several basic questions which cannot currently be addressed. For example, presuming we can routinely accomplish the clinical-scale expansion of tumor-reactive γδ-T cells from patients with prostate cancer. Will infusing supra-physiological numbers of these γδ-T cells restore or augment innate immune responses against tumors, thus moderating tumor growth or progression? Can these γδ-T cells be utilized alone or will they best be utilized in conjunction with standard hormone, chemotherapy or radiation-based treatments? Only properly designed future clinical trials – based largely upon these such findings reported here – will be able to adequately address these issues.
Figure 1. Composition of ex-vivo expanded apoptosis resistant γδ-T cells with respect to express TCR usage. Total γδ-T cells were first isolated from PBMC cultures by immunomagnetic column separation. Purified cells were cultured for 24 hours prior to analysis to allow mAb used for sorting to dissociate. Anti-Vδ1 and Vδ2 mAb conjugated with FITC and PE respectively as well as anti-CD3 mAb with APC were incubated with the cells. After being washed, the cells were analyzed by FACS. PI negative events were gated for analysis to exclude non-viable cells. Percentage of cells in the corresponding quadrant locations are shown. Result are representative of experiments performed at least three separate times using PBMC derived from healthy donors.
Figure 2. Antitumor activity of Vγ9Vδ2 γδ-T cells against human prostate cancer cell lines. PBMC were isolated from healthy donors then cultured as described above. After 14 days, apoptosis-resistant γδ-T cells were sorted to high purity utilizing the Miltenyi AutoMACS immunomagnetic cell separator. Human prostate cancer cell lines DU145, PC-3 and LNCaP were first labeled with 51Cr, then incubated with γδ-T cells or control αβ-T cells. After a 4 hour incubation at the indicated E:T ratios, supernatants were removed to determine 51Cr release in CPM. Data are expressed as the mean percent specific target lysis (± SD) of triplicate determinations. These studies are representative of experiments performed at least 3 other times.
Figure 3. Antibodies to CD18 and CD54/ICAM inhibit Vγ9Vδ2 γδ-T cell-mediated tumor lysis. Cytotoxicity assays were performed in the presence of anti-TCR-Vδ2, anti-TCR-Vγ9 or anti-TCR-γδ mAbs (or corresponding isotype controls). Effector γδ-T cells were isolated as described and co-cultured with $^{51}$Cr-labeled cell lines A) DU-145 or B) PC-3 at an E:T ratio of 10:1. Similarly in C), effector γδ-T cells were co-cultured with $^{51}$Cr-labeled PC-3 cells with the addition of mAb against CD54/ICAM-1 or CD18 (or isotype control). Relative tumor specific lysis (+ SD of triplicate determinations) in the presence of each specific antibody was calculated by comparing with its corresponding isotype control. Studies are representative of experiments performed at least 3 other times.
Figure 4. Cytolysis of prostate cancer cells by Vγ9Vδ2 γδ-T cells involves the perforin/granzyme pathway. Standard 4 hour cytotoxicity assays were performed using ex vivo expanded Vγ9Vδ2 γδ-T cells as effector cells and ⁵¹Cr-labeled DU145 cells as target cells (E:T ratio, 10:1)).

a) Effector γδ-T cells co-cultured with ⁵¹Cr-labeled cell DU145 in RPMI;
b) ⁵¹Cr-labeled cell DU145 cultured without γδ-T cells;
c) Effector γδ-T cells co-cultured with ⁵¹Cr-labeled cell DU145 in the absence of Ca²⁺ (depleted by the addition of EGTA);
d) Effector γδ-T cells co-cultured with ⁵¹Cr-labeled cell DU145 with Ca²⁺ replaced.
REFERENCES


