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Towards therapeutic stimulations of $\gamma\delta$ T cells

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Most vaccine strategies are designed to elicit adaptive immune responses to a variety of microbial or tumor-associated antigens. These immune responses are predominantly mediated by $\alpha\beta$ T cells, B cells and antibodies. Nevertheless, approximately 1–5% of human peripheral blood lymphocytes possess the surface $\gamma\delta$ T-cell receptor, predominantly expressing the V γ 9V δ 2 variable segments. V γ 9V δ 2 T lymphocytes recognize nonpeptidic antigens (NpAgs) generated by the DOXP (many eubacteria, algae, plants, apicomplexa) and mevalonate (eukaryotes, archaeobacteria and certain eubacteria) pathways of isoprenoid synthesis. NpAgs are molecules structurally distinct from the typical peptidic antigens that interact with $\alpha\beta$ T-cell receptors. Also, the recognition of NpAgs by V γ 9V δ 2 T cells does not require classical antigen processing and MHC class I or II presentation. It is believed that this pattern of recognition allows for a rapid primary immune response to antigen challenge, particularly by infectious agents.^{1,2}

In addition to NpAgs, it has also been demonstrated that certain nitrogen-containing bisphosphonates (N-BPs) such as pamidronate disodium or zoledronic acid are potent stimulators of V γ 9V δ 2 T cells.^{3,4} Originally, Kunzmann and colleagues demonstrated that the anti-tumor effect of pamidronate on myeloma cells was correlated with its ability to elicit $\gamma\delta$ T cells *in vitro* using bone marrow from patients with multiple myeloma.⁵ The investigators also reported that V γ 9V δ 2 T cells could be detected in the bone marrow of patients with multiple myeloma, and that cytoreduction of these cells permitted the outgrowth of malignant myeloma cells.⁵ In 1995, Choudhary *et al.*⁶ showed unequivocally that $\gamma\delta$ T cells infiltrate renal cell carcinomas (RCCs) and the infiltrating $\gamma\delta$ T cells are cytotoxic for autologous tumors. Recently, these studies were confirmed and extended by Viey *et al.*⁷ For studying $\gamma\delta$ T cells in cancer patients, we have developed sensitive, non-radioactive assays for

assessing the capacity of V γ 9V δ 2 T cells to kill cancer cells *in vitro* (Figures 1 and 2). These assays are based on measuring a) the retention of a calcein fluorophore by viable cells (*see below*), b) the assessment of caspase activities (data not shown) and c) the release of nonradioactive lanthanides, specifically ⁶²Sm, ⁶³Eu and ⁶⁵Tb by lanthanide-labeled dead cells (*data not shown*).

A common side effect with administration of zoledronic acid, and frequently with other bisphosphonate drugs, is the development of fever, myalgias, nausea, and other flu-like symptoms, 24–48 hours later. This is most common with the first dose, and is less frequently observed with subsequent doses. This has been attributed to a burst of inflammatory cytokines, in particular TNF- α and IL-6, detectable in the serum of treated patients.^{8,9} *In vitro*, PBM-Cs isolated from normal donors, secrete large amounts of IFN γ and TNF- α , when incubated with increasing doses of either pamidronate or zoledronic acid. These changes are associated with $\gamma\delta$ T-cell activation, as measured by down-regulation of the $\gamma\delta$ TCR. In addition, lower concentrations of either pamidronate or zoledronic acid, in combination with IL-2 lead to expansion of $\gamma\delta$ T cells; higher concentrations lead to decreased expansion and increased apoptosis, as measured by Annexin V surface staining. This increase in $\gamma\delta$ T cell death is likely due to over-stimulation, or activation-induced cell death, as evidenced by the decrease in cell TCR frequency. In addition, part of this effect may be mediated by the induction of apoptosis of monocytes as antigen-presenting cells as a direct effect of the aminobisphosphonates on this population.¹² These findings, together with the clinical observation that cytokine-mediated side effects are ameliorated with subsequent dosing, suggest that higher doses of the aminobisphosphonates may, in fact, lead to over-activation of $\gamma\delta$ T cells *in vivo*. It has been reported¹³ that the peak serum concentration with the typical dose of 4 mg intra-

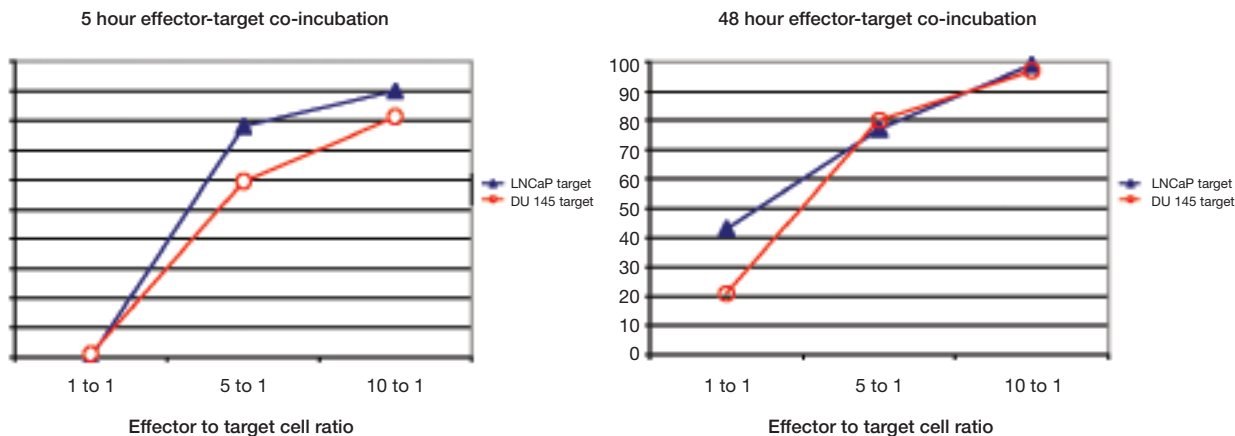


Figure 1. Destruction of prostate cancer cells by the $V\gamma 9/V\delta 2$ T-cell line A1/C2 *in vitro*. Prostate cancer cells lines (LNCaP or DU145) were plated in complete RPMI medium and allowed to adhere to tissue culture wells overnight in a CO₂ incubator (37°C). The next day, nonadherent $V\gamma 9V\delta 2$ T-cell effectors or media were added. In this experiment, the plates were incubated for 5 or 48 hours. Effector cells or media were carefully removed and plates were gently washed with PBS. Viability was determined using the LIVE/DEAD™ Viability/Cytotoxicity Assay for Animal Cells using the SPECTRAmax GEMINI EM Fluorescence Microplate Reader (Molecular Devices, Sunnyvale, CA). In brief, 5 μ M Calcein AM (a nonfluorescent, esterase substrate that is cleaved by cytoplasmic esterases upon diffusion into live cells, releases a calcein fluorophore that is retained in viable cells) was added to all wells including the blanks. After a 4-h incubation (37°C), relative fluorescence units (RFU) that are directly proportional to the number of live tumor cells were read from the bottom of plates using the well scan feature at the excitation wave length of 488 nm (emission – 525 nm; cut off filter – 515 nm). The percent specific cytotoxicity was calculated by $[(RFU \text{ for tumor cells alone} - RFU \text{ for tumor cells incubated with effectors}) / (RFU \text{ for tumor cells alone} - \text{media RFU})] \times 100$. The A1/C2 $V\gamma 9V\delta 2$ T-cell line was derived from normal human PBMCs using the method described by Fisch *et al.*^{10,11}

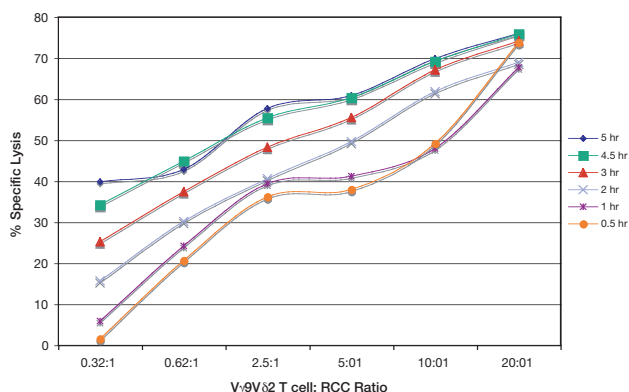


Figure 2. $V\gamma 9V\delta 2$ T-cell-mediated cytotoxicity against renal cancer cells. Lysis of renal cancer cells (RCC 786-0) *in vitro* by $V\gamma 9V\delta 2$ T cells was measured using the LIVE/DEAD™ Viability and Cytotoxicity Assay as described in the legend to Figure 1.

venous dose, administered over 15 minutes, is 264 ± 86 ng/mL ($\sim 1 \mu$ M), a dose at which we have observed increased apoptosis *in vitro*. An *in vivo* dose titration study is necessary to identify an appropriate dose to activate and expand $\gamma\delta$ T cells *in vivo* without resulting in overactivation and apoptosis of these cells.

Recently, we have shown that intravenous administration of N-BPs or pyrophosphomonoester drugs combined with low doses of IL-2 induces a large pool of CD27⁺ and CD27⁻ effector/memory $V\gamma 9V\delta 2$ T cells

in the peripheral blood.¹⁴ Moreover, we have observed anecdotal cases of patients with hormone-refractory prostate cancer, who have been treated with pamidronate or zoledronic acid for palliative purposes and who have had evidence of a PSA decline and/or stabilization in the absence of other active therapies.¹⁵ Several clinical trials focused on $\gamma\delta$ T-cell activation *in vivo* in patients with various cancers are currently in progress. The findings from these trials, their future combinations with conventional therapies, and the wealth of information that has been learned particularly over the last decade are likely to further improve the armament of clinical oncologists as well as specialists in infectious diseases.¹⁶⁻¹⁸

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