Towards therapeutic stimulations of γδ T cells

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 αβ T cells, B cells and antibodies. Nevertheless, approximately 1–5% of human peripheral blood lymphocytes possess the γδ T-cell receptor, predominantly expressing the Vγ9Vδ2 variable segments. Vγ9Vδ2 T lymphocytes recognize nonpeptidic antigens (NpAgs) generated by the DOXP (many eubacteria, algae, plants, apicomplexa) and mevalonate (eukaryotes, archaeabacteria and certain eubacteria) pathways of isoprenoid synthesis. NpAgs are molecules structurally distinct from the typical peptidic antigens that interact with αβ T-cell receptors. Also, the recognition of NpAgs by Vγ9Vδ2 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. 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 Vγ9Vδ2 T cells. Originally, Kunzmann and colleagues demonstrated that the anti-tumor effect of pamidronate on myeloma cells was correlated with its ability to elicit γδ T cells in vitro using bone marrow from patients with multiple myeloma. The investigators also reported that Vγ9Vδ2 T cells could be detected in the bone marrow of patients with multiple myeloma, and that cyto-redution of these cells permitted the outgrowth of malignant myeloma cells. In 1995, Choudhary et al. showed unequivocally that γδ T cells infiltrate renal cell carcinomas (RCCs) and the infiltrating γδ T cells are cytotoxic for autologous tumors. Recently, these studies were confirmed and extended by Viey et al. For studying γδ T cells in cancer patients, we have developed sensitive, non-radioactive assays for assessing the capacity of Vγ9Vδ2 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 60Sm, 66Eu and 65Tb by lanthamide-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. In vitro, PBMCs isolated from normal donors, secrete large amounts of IFNγ and TNF-α, when incubated with increasing doses of either pamidronate or zoledronic acid. These changes are associated with γδ T-cell activation, as measured by down-regulation of the γδ TCR. In addition, lower concentrations of either pamidronate or zoledronic acid, in combination with IL-2 lead to expansion of γδ T cells; higher concentrations lead to decreased expansion and increased apoptosis, as measured by Annexin V surface staining. This increase in γδ 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. 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 γδ T cells in vivo. It has been reported that the peak serum concentration with the typical dose of 4 mg intra-
dose titration and CD27- effector/memory Vγ9T-lymphocyte γ10 to 15 to 1T-lymphocyte γ10 to 15 to 1 in patients with various cancers are currently incubated (37°C). The next day, nonadherent Vγ9Vδ 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 Moreover, we have observed % Specific Lysis

![Graph showing % Specific Lysis vs. Effector to target cell ratio](image)

of prostate cancer cells by the Vγ9/Vδ2 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 Vγ9Vδ2 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

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Moreover, we have observed increased apoptosis in vitro. An in vivo dose titration study is necessary to identify an appropriate dose to activate and expand γδ 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 Vγ9Vδ2 T cells in the peripheral blood. 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.

Several clinical trials focused on γδ 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.

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**Figure 1.** Destruction of prostate cancer cells by the Vγ9/Vδ2 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 Vγ9Vδ2 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 Moreover, we have observed % Specific Lysis

**Figure 2.** Vγ9Vδ2 T-cell-mediated cytotoxicity against renal cancer cells. Lysis of renal cancer cells (RCC 786-0) in vitro by Vγ9Vδ2 T cells was measured using the LIVE/DEAD™ Viability and Cytotoxicity Assay as described in the legend to Figure 1.

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**References**


