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Title of Presentation: Toll-like receptor agonists as therapy for childhood acute leukemia

Abstract

Despite achieving high rates of first complete remissions, relapsed B-cell precursor (BCP) acute lymphoblastic leukemia (ALL) remains the 5th most common childhood malignancy. BCP ALL cells are poor stimulators of T cell-mediated immune responses due to their low expression levels of co-stimulatory molecules. However, the success of hematopoietic stem cell transplants in ALL has shown that donor-derived immune activity can provide protection against leukemia progression. Developing new strategies that enhance the anti-leukemia immune responses by overcoming the immune unresponsiveness to leukemic blasts may contribute significantly to therapy for childhood ALL.

The ligation of Toll-like receptors (TLR) leads to activation of innate immune responses. This is sufficient for initiating adaptive immune responses by TLR-expressing cells, including leukemic blasts. Previously, we have established long-term protection against BCP ALL following administration of a TLR9 agonist, immunostimulatory CpG ODN (oligodeoxynucleotides containing CpG motifs), in a transplantable syngeneic BCP ALL model. The effect mediated through other TLRs, however, remains to be elucidated. In this study, we investigated the ability of the following TLR agonists to induce anti-leukemia activity: TLR2/1 agonist Pam(3)CSK(4) (Pam 3), TLR3 agonist polyinosinic:polycytidylic acid (poly I:C), and TLR7/8 agonist resiquimod (R848).

First, we showed that the primary BCP ALL cells harvested from our spontaneous BCP ALL mouse model express all the relevant TLRs for the agonists utilized in this study indicating their potential for responding directly to the stimulation by agonists. In functional *in vitro* studies, TLR2/1 stimulation significantly increased the expression level of CD40 on BCP ALL cells, 3-fold higher than that of TLR9, while neither TLR3 nor TLR 7/8 stimulation induced the expression on cells ($p < 0.0001$). Moreover, only Pam 3 exerted a strong cytotoxic effect on primary BCP ALL cells by significantly reducing the cell viability ($p < 0.0001$) and poly I:C elicited an opposing effect by enhancing the survival of cells ($p < 0.002$). Likewise, Pam 3 induced the strongest lymphocyte-mediated cytotoxicity against primary BCP ALL cells in a dose-dependent manner *in vitro*. Finally, the *in vivo* activity of TLR agonists was evaluated using a syngeneic adoptive transfer model. While CpG ODN or poly I:C treatment significantly depleted the BCP ALL cell population in the leukemia-bearing mice by day 21 post-challenge, a marked increase in cell number was observed in the blood ($p < 0.002$) and spleen ($p < 0.0001$) but not in bone marrow of mice following R848 treatment. In contrast to its *in vitro* efficacy, Pam 3 treatment fail to inhibit the expansion of BCP ALL cells. Despite inhibiting the expansion of the BCP ALL cell population early in the course of study, poly I:C treatment did not confer a survival benefit. Consequently, mice treated with PBS-control or TLR agonist other than CpG ODN all died of leukemia by week 6-post challenge.

The results of this study indicate that these TLR agonists generate anti-leukemia immune activity in varying strength *in vitro*. The *in vivo* efficacy of TLR agonists do not correlate with the strength of *in vitro* direct actions recapitulating the fact that the TLR agonist-induced protective activity is mediated by antigen non-specific immune stimulation. Taken together, these results further support the potential for CpG ODN as the most effective TLR-based novel therapy for childhood ALL to date. Work is ongoing to define the mechanisms of this process and identify the key mediators of CpG ODN-induced anti-leukemia immune responses.