Manipulation of Immune Response through IL-2 in B-Cell Acute Lymphoblastic Leukemia

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Introduction:

Interleukin-2 (IL-2) therapies are promising for treating cancer because they help boost the immune system. However, these therapies have several problems that limit their effectiveness. First, the benefits of IL-2 are often short-lived, providing only temporary relief for many patients. High doses can also cause serious side effects, including flu-like symptoms and capillary leak syndrome, where blood vessels leak fluid into tissues, leading to severe complications such as low blood pressure and organ issues[1]. It can also cause cardiovascular and respiratory issues, renal dysfunction, gastrointestinal symptoms, neurological effects, skin reactions, blood cell count changes, and increased infection risk[1]. Additionally, IL-2 can unintentionally stimulate cells that suppress the immune response, reducing its effectiveness. The environment around tumors can also make it harder for IL-2 to work well[1]. Tumors often create conditions that make immune cells less effective or hinder their ability to attack cancer cells[2]. Moreover, the complexity of immune system interactions can complicate the therapy's success. These factors make IL-2 therapy less effective than hoped, showing a need for more research to improve its results and manage its side effects[2]. Advances in understanding how to better target IL-2 and reduce its adverse effects are crucial for enhancing its therapeutic potential. Therefore, this work aims to investigate how induction of IL-2 could potentially promote the growth of cancer cells instead of promoting immune responses.

Materials and Methods:

Allogeneic T cells were thawed and cultured in a T-25 flask. The cells were washed, the supernatant removed, and the pellet resuspended in complete media (RPMI 1640+10% FBS+20mM HEPES). To determine the effect of different seeding densities (50k, 100k, 150k, 200k) on cell doubling time, the B-ALL (acute lymphoblastic leukemia) NALM6 cells were counted and imaged. The NALM6 cells were engineered to express Firefly Luciferase for the downstream luciferase assay. To study the effect of varying IL-2 concentrations on B-ALL cell proliferation with T cells, we co-cultured them at different concentrations (20, 4, 0.2, and 0 ng/mL). 3E4 T cells were seeded with 1E4 leukemia cells in a 96-well U-bottom plate. After pooling, the B-ALL cells were transferred to a conical tube, rinsed with PBS, and centrifuged. The T cells were removed, washed, and spun down. A 3:1 T to B cell coculture suspension was prepared. The cells were centrifuged (300g for 5 minutes), and the conditioned media from wells

with T cells and leukemia cells were collected, then frozen at -80°C. Cells were washed with PBS, then 20 µL of lysis buffer was added to release intracellular luciferase. Using a repeater pipette, 100 µL of luciferin was added to the lysate and shaken for 5 minutes. Luminescence was measured with a Tecan Genios Pro plate reader at 1000 ms to assess B-ALL cell proliferation. The luminescence values from the co-culture were compared with B-ALL controls without IL-2 to determine the effect of IL-2.

Results and Discussion:

The impact of initial seeding density on the proliferation dynamics of B-ALL cells (leukemia cells) reveals a positive correlation between the initial number of seeded cells and the subsequent expansion rate. Higher seeding densities result in significantly accelerated proliferation, indicating that the initial concentration of cells is crucial in determining the overall growth of the cell population (Figure 1).

Following the co-culture of B-ALL (B-cell acute lymphoblastic leukemia) cells with T cells, it was observed that IL-2 promoted B cell proliferation. The relative B-ALL cell numbers in the co-culture system were assessed at varying IL-2 concentrations and normalized to the pure B-ALL control. In the normalized pure B-ALL population, the highest relative cell counts were observed at IL-2 concentrations of 20 ng/mL and 4 ng/mL (Figure 2 and 3).

Additionally, IL-2 was added to the co-cultures at various concentrations to assess cytokine release, and the levels were measured. The data indicated that the highest release occurred at an IL-2 concentration of 4 ng/mL, compared to the control group without B-ALL cells (Figure 3).

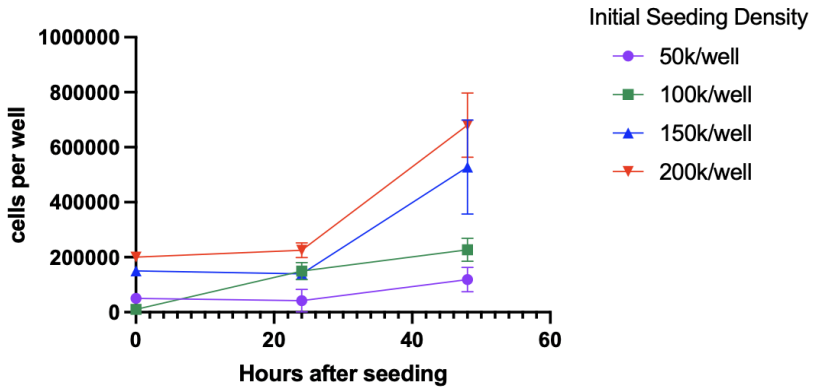

Figure 1: Varying seeding densities based on cell doubling time and expansion rate.

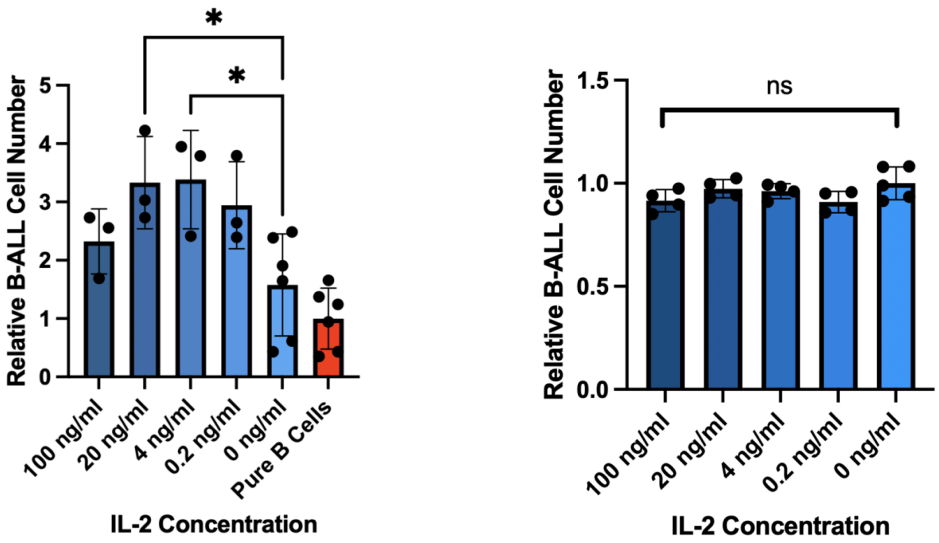

Figure 2: (Left) Relative B-ALL cell number in the co-culture system with T-cells with varying concentrations of IL-2. The values are normalized to the pure B-ALL control. (Right)

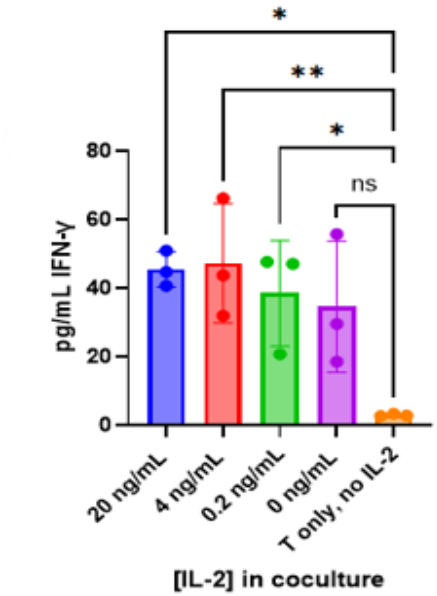

Figure 3: Co-culture system of cytokine release in different IL-2 concentrations.

Conclusion:

The findings from our experiments highlight several critical insights into the proliferation dynamics of B-ALL cells and the role of cytokines in modulating these processes. Firstly, our investigation into the impact of initial seeding density on B-ALL cell proliferation demonstrates a clear positive correlation between the initial cell concentration and the subsequent expansion rate. This observation underscores the importance of initial cell density in determining the growth kinetics of leukemia cells, suggesting that higher seeding densities significantly accelerate proliferation. In addition to seeding density, our studies also elucidate the role of IL-2 in promoting B-ALL proliferation within a co-culture system of B-ALL cells and T cells. Our results suggest that these specific cytokine levels are particularly effective in stimulating B cell proliferation, thereby highlighting the potential of IL-2 as a modulatory agent in B-ALL cell growth. Our data emphasizes the cytokine's significant impact on the immune response. In conclusion, these findings collectively suggest that both the initial seeding density of B-ALL cells and the presence of certain cytokines, like IL-2, are crucial for the growth of B-ALL cells. In conclusion, these results suggested that in an immunosuppressive environment where the cytotoxic T lymphocytes are exhausted, the induction of IL-2 can trigger the proliferation of T cells and the release of more IL-2. This result ultimately promotes the growth of B-ALL cells through the feedback loop of IL-2 production.

References:

1. Rosenberg, S. A., & Restifo, N. P. (2015). Adoptive cell transfer as personalized immunotherapy for human cancer. *Science*, 348(6230), 62-68. https://doi.org/10.1126/science.aaa4967
2. Buchbinder, E. I., & Desai, A. (2021). CTLA-4 and PD-1/PD-L1 inhibitors in cancer treatment. *The Lancet*, 398(10301), 1005-1016. https://doi.org/10.1016/S0140-6736(21)01845-9