SynthImmunol_NMU Notebook No.1

Time: 2024.07.01-2024.07.07

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1. **Experiment:** The cytotoxic activity detected by LDH
2. **Time:** 2024.07.01-2024.07.07
3. **Member:** Song Zhang, Xudong Tang, Hanyue Liu, Xiaoyuan Chen, Xinyu Zhu, Yinran Luo
4. **Material:** Microplate reader, LDH assay kit (Promega), 96-well plate, Pipette, αCD19-CAR cell line, αCD20-CAR cell line, NK cell, RPMI-1640 medium (10-15 mL per culture flask, Gibco), FBS (Gibco)
5. **Method:**
   (1) Cell preparation:
   ① Seed cells into culture dishes with the appropriate medium. Incubate at 37°C with 5% CO_2 for 2 days. Change to a fresh medium the evening before the experiment.
   ② Adjust the density of effector cells to 1.0×10^4 cells/mL and tumor cells to 2.0×10^3 cells/mL.
   (2) Cytotoxicity assay:
   ① Prepare a 96-well round-bottom plate for the cytotoxicity assay. Conduct experiments at E:T ratios of 5:1 and 2:1, with replicates per ratio. Add effector and target cells to each well, with a total volume of 100 μL per well.
   ② Set up control wells: Natural release control wells with the same number of effector cells as in the experimental wells. Maximum release control wells with the same number of target cells, adding medium to achieve a final volume of 90 μL per well. Natural release control wells for target cells, adding medium to achieve a final volume of 100 μL per well. Background control wells with 100 μL of CAR-NK cells.
   ③ Add 10 μL of sterile ultrapure water to the spontaneous release control wells for both effector and target cells.
   ④ Incubate the plate at 37°C with 5% CO_2 for 4 hrs.
   ⑤ Add 10 μL of lysis buffer to the maximum release control wells and incubate for 45 mins.
   ⑥ Centrifuge at 250 × g for 3 mins. Transfer 50 μL of supernatant from each well to a corresponding flat-bottom plate. Add 50 μL of reaction substrate to each well and incubate in the dark at room temperature (25°C) for 30 mins. Once the color deepens, add 50 μL of stop solution to each well and gently mix the plate.
   ⑦ Using a microplate reader, Measure the absorbance at 490 nm and 680 nm. Subtract the absorbance value at 680 nm (background signal) from the absorbance at 490 nm (D).
   ⑧ For accurate calculations, subtract the background control average value from the experimental average, the effector cell spontaneous release control value, and the target cell spontaneous release control value.
   ⑨ Calculate the NK cell killing rate (%) using the following formula:
   NK killing rate (%)=Target cell lysis OD value−Target cell spontaneous release OD value Experimental OD value−Effector cell spontaneous release OD value−Target cell spontaneous relea se OD value−Background control average value×100%
6. **Result:**

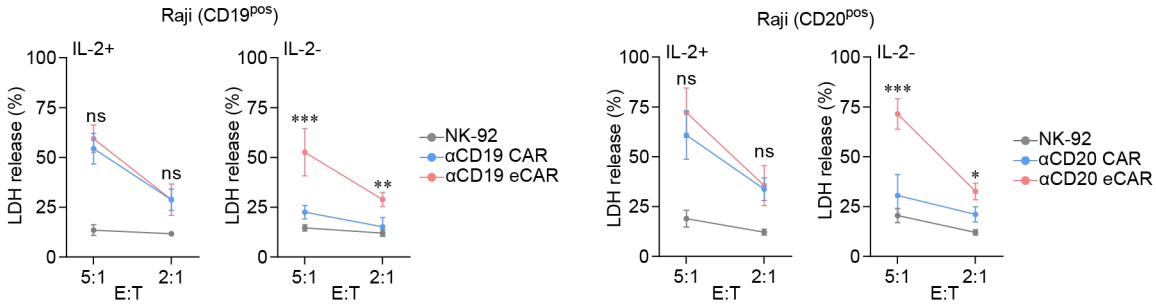

Fig.1 The cytotoxic activity detected by LDH experiments

Co-culturing with CD19-positive Raji cells without exogenous IL-2, the percent of LDH release of NK92 cells transduced with αCD19 eCAR sequence was significantly higher than NK92 cells without transduction or transduced with CAR sequence, which was observed at effector-to-target (E:T) ratio of both 5:1 ($P < 0.001$) and 2:1 ($P < 0.01$). Similar results were observed when NK92 cells transduced with αCD20 eCAR sequence were co-cultured with CD20-positive Raji cells ($P < 0.001$ for E:T ratio of 5:1 and < 0.05 of 2:1).