Natural Killer Cell Dynamics and Cytotoxicity in Droplet Microfluidics
Sayalee Potdar, Saheli Sarkar, Pooja Sabhachandani, Tania Konry
Department of Pharmaceutical Sciences, School of Pharmacy, Bouve School of Health Sciences

ABSTRACT
Natural Killer (NK) cells are an essential component of innate immune response against pathogens and tumor cells. The cytotoxic nature of NK cells is attributed to the release of perforin and Granzyme B that induce apoptosis in target cells. Existing cytotoxicity assays are done with a mixed population of NK and target cells that fail to provide a comprehensive overview of the dynamic nature of NK cell synaptic contacts or the mechanism of target death. Microfluidic single-cell analysis allows us to visualize NK-target cell interaction, provide greater control of cell pairing and model various effectors to target ratios in the same experiment. We have assessed NK cell functionality at single-cell level using Non-Hodgkin’s (NHHL) B cell lymphoma line (SUDHL-10) and patient-derived NHL cells as target in droplet microfluidic platform. Our findings indicate that activated NK cells efficiently mediated SUDHL-10 cell death within 1-2 hours and also killed patient-derived NHL cells to a significant degree. Varying effector to target ratio did not significantly increase target cell death.

GOALS
To develop an in vitro single-cell analysis platform that permits preclinical assessment of the interactions between SUDHL-10 cells and allogeneic NK cell-based therapies. This would enable quantitative assessment of activation, effector functions and the therapeutic efficacy of immunomodulatory agents at single cell level.

RESULTS

Response of patient-derived lymphoma

Perforin secretion

Target (SUDHL10) cells were incubated in droplets with unconditioned [untreated], conditioned media (CM) from IL2-treated (CM-IL2) or IL2-untreated (CM-No IL2) NK cells. Target cell death was determined in the absence of NK cells over 4hrs.

Cytokine secretion by NK cells
The mechanism of NK mediated target killing was investigated in the presence of inhibitors such as calcium chelator EGTA, which interferes with the perforin pathway and Brefeldin, which inhibits cytokine secretion.

DROPLET-BASED SINGLE CELL CYTOTOXITY

NK cell dynamics in droplets. (A) Schematic of droplet microfluidic device. (B) Image of microfluidic droplet generation. (C) Contact between NK (unlabeled) and target SUDHL10 (Calcein AM labeled) cell in droplet. Loss of target cell viability was observed at 24hrs. (D) Target cell remains viable despite contact with NK cell. Scale bar: 50µm. (E) Quantification of target SUDHL10 cell death due to contact-dependent and – independent mechanisms in droplets. (F) Single NK cell lyses target cells serially. (NK cell: Hoechst labeled; target cells: labeled with Calcein AM and Hoechst).

IMPACT
Existing cytotoxicity assays are done with a mixed population of NK cells and target cells but this does not provide information about the heterogeneity of either NK or target cells or the mechanism of target death. Single cell analysis in a dynamic, time-dependent manner can provide insights into the heterogeneity of NK cell function, exact killing mechanisms and changes in NK cell interactions. Our microfluidic system is a robust, high-throughput method for functional evaluation of effector immune functions and enables a controlled environment to characterize cellular interactions. This platform can further allow interaction of cell pairs at various effector to target ratios in the same experiment, as shown by the serial interactions between NK and target cells. Serial killing is an important mechanism of target elimination by a small subset of NK cells. In future, this single-cell cytotoxicity assay could help assess the efficacy of NK-based immunotherapies prior to clinical application.

FUTURE WORK AND CLINICAL RELEVANCE
- We plan to sort NK cells based on contact-vs. non-contact mechanisms and subsequently use genomic profiling techniques to identify differences in molecular mechanisms between the two subsets of NK cells.
- To use the microfluidic droplet system to characterize interaction of target cells with transgenic chimeric antigen receptor (CAR)-NK cells and understand the cytotoxicity mechanisms.
- To use the microfluidic platform in a pre-clinical setting and assess optimal immunotherapies for cancer patients based on patient responses to immune cells at single-cell level.

REFERENCES

ACKNOWLEDGMENT
We would like to acknowledge the grant NIH/NCI fund R21 [R011-014] given to Dr. Konry and Dr. Andrew Evans, Dr Ravi Dashnamothry and Dr. Afshin Beheshti, our collaborators from Tufts Medical School, for providing us SUDHL10s cells. We would also like to thank our M.S students Ms. Sneha Pawar, Ms. Chaitra Belur, Ms. Himali Shroff and Mr. Sai Mynampati for their assistance in device fabrication and experimental setup.