THE CYTOTOXCITY ACTIVITY OF *IN VITRO* ISOLATED AND EXPANDED CYTOTOXIC T-LYMPHOCYTES AND NATURAL KILLER CELLS IN BLADDER CANCER

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The expanding roles of the immune system in tumourigenesis have established immunotherapy as a potential mainstream cancer therapeutic modality. *Ex vivo* expanded and activated cytotoxic T-lymphocytes (CTLs) and natural killer (NK) cells have been found to be efficacious in the treatment of various types of cancers. One of the biggest limitations is the ability to generate and store cytotoxic immune cells in larger numbers without losing its cytotoxicity. Consequently, we evaluated the *in vitro* cytotoxic activity of freshly cultured and cryopreserved CTLs and NK cells that were expanded *in vitro*. We also compared the synergistic cytotoxic activity of CTLs and NK cells in combination. The cytotoxic activity was measured in bladder cancer cell lines, EJ28 (invasive) and RT112 (minimally-invasive). All experiments were run in three replicates. The cellular phenotype of the isolated and expanded effector cells was characterised using flow cytometry. MTT assay was performed to assess the dose- and time-dependent cell-mediated cytotoxic activity in the bladder cancer cells. An effector to target ratio of 1:1, 2:1, 5:1, 10:1 and 20:1 was tested after 4 h, 12 h and 24 h incubation. The fresh *in vitro* expanded effector cells had a high percentage of cell viability and expressed cytotoxic markers CD8+ and CD56+ in the CTL and NK cell cultures, respectively. Although the expansion capacity of the cryopreserved cells was limited, the expression of the functional markers and cytotoxic activity of these effector cells were maintained. All the effector cells exhibited significant cytotoxic activity at the effector to target ratio of 5:1 at 4 hours of co-culture. This was confirmed through the real-time observation of the morphological changes of the cells using an inverted phase contrast and time-lapse confocal microscope. The *ex vivo* generated CTLs and NK cells appear to retain their functionality, especially in recognizing their allogeneic target and thus, serve as a foundation to build on for future therapeutic applications.