MAINTAINING CD4/CD8 RATIO AND TH1-CTL SUBSETS OF CHIMERIC ANTIGEN RECEPTOR (CAR)-T CELLS IN SERUM-FREE CULTURE CONDITIONS

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Chimeric antigen receptor (CAR) T cells therapy is a promising strategy that significantly controlled the progress of cancer diseases. CAR-T cells could kill cancer cells through cellular immune response; therefore, CD8+ cytotoxic T cells are critical for CAR-T cell therapy. However, recent papers reported that CD4+ T helper cells were important for the response and maintenance of CAR-T cells *in vivo*. Here, we developed a serum-free CAR-T cell preparation process that maintained the T cell population and controlled the T cell subsets. The CD4+ and CD8+ T cell population in CAR-T cells were maintained at averagely 59.4 % and 34.6%, and the major T cell subsets were Th1 cells and cytotoxic T lymphocytes (CTLs), implying the potentially high cellular immune response. To verifying whether the prepared CAR-T cells were exhausted, the expression of several immune checkpoint markers was determined. Of interest, only less than 20% of CAR-T cells at endpoint were PD-1+ or CTLA4+, but more than 40% of CAR-T cells at the endpoint were TIM-3+, implying most CAR-T cells were not exhausted. These CAR-T cells produced more than 1 ng/mL of IFN-γ in the response to the antigen. Altogether, CAR-T cells could be prepared in our serum-free process in the controlling of T cell subsets, leading to potential high therapeutic potency.
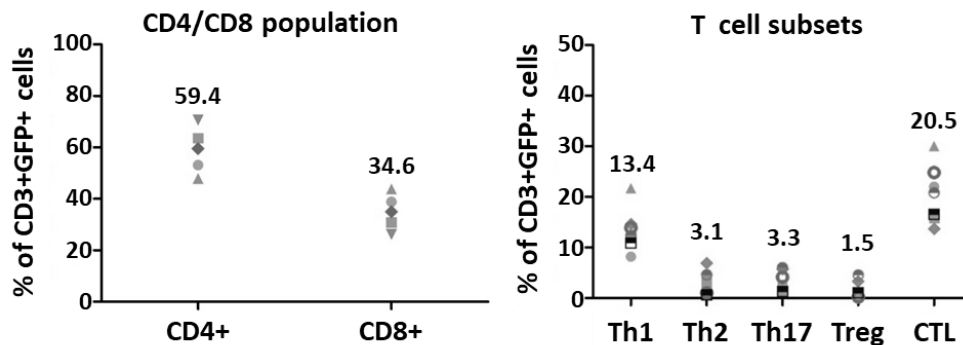
Figure 1 – The major cell subsets of CAR-T cells expanded in the serum-free culture condition for 6 days were Th1 and cytotoxic T lymphocytes (CTLs) that participated in the cellular immune response.