BIOPROCESS OPTIMIZATION FOR THE EXPANSION OF EARLY MEMORY T CELLS IN SERUM-FREE CONDITIONS

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The emerging field of adoptive transfer of T lymphocytes holds a tremendous promise for the treatment of advanced therapy-resistant tumors[1]. Preclinical and animal studies have suggested that the clinical success rate of immunotherapy can be linked to T cell-related attributes such as longevity, proliferative potential and metabolism. In particular, less differentiated T lymphocyte subsets such as T stem and central memory cells show increased antitumor activity compared to effector T cells due to higher in vivo expansion rates and longer persistence in the recipient organism[2]. The nature of the infused T cell population is largely determined by the protocols used during the expansion. The vast majority of the currently reported methods for T cell culture are based on the use of high doses of IL-2 to maximize the growth rate of the cells, at the expense of vast differentiation towards the short-lived effector phenotype. Recently, several reports have identified the molecular mechanisms behind the progressive differentiation stages driving the development of T memory and effector subsets[3]. However, the conditions used for the formation and expansion of T memory subsets in vitro involve the use of poorly defined human serum components. Additionally, no real investigation of the physicochemical environment and engineering parameters for lymphocyte culture under serum free conditions has been reported.

In order to develop a process that could generate T cells with improved antitumor efficacy in more defined conditions, we first identified the critical medium components capable of substituting the addition of serum. Secondly, we screened for signaling agonists and inhibitors in order to influence the pathways that drive differentiation towards memory or effector phenotypes. Lastly, a DoE approach was performed to evaluate the effect of growth enhancers and physicochemical variables to maximize the lymphocyte expansion rate.
Our results demonstrate a valuable alternative to serum-supplemented media to generate large number of T cells with an early memory phenotype (CCR7+ CD27+) starting from unfractionated human CD3+ T lymphocytes. Moreover, this approach leads to growth rates comparable to standard protocols, with the advantage of reduced costs and variability linked to the use of human serum or platelet lysates.

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