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Introduction

The efficiency of an ongoing immune response depends on the cytokines produced by T helper types 1 (Th1) and 2 (Th2), as well as T cytotoxic types 1 (Tc1) and 2 (Tc2). Patients with cancer may experience reduced T-cell-mediated immune system activity due to dysregulated growth of one or both subsets. In the current investigation, we looked into the possibility of such dysregulation in young patients with acute lymphoblastic leukaemia (ALL) [1]. CD4⁺ cells can be divided into various categories that each release distinctive clusters of

conjugate with a poorly stained FITC conjugate that stains the same cell population due to the spectrum overlap of AmCyan into the FITC channel [7].

Processing of samples

Cell therapy development, manufacture, and application continue to face substantial challenges with regard to in-process monitoring and control of biomanufacturing work flows [8,9]. To guarantee consistent and predictable safety, efficacy, and potency of clinical products, new process analytical methods must be developed to identify and manage the crucial process parameters that influence ex vivo cell growth and differentiation. There are various categories of functional indicators that have similar ideal stimulation conditions [10]. For instance, the majority of cytokines, including as IL-2, IL-4, IL-5, IL-13, IFN, MIP-1, and TNF, peak between 6 and 12 hours after stimulation. Although CD154 keeps this level of expression for up to 24 hours (26), CD107 and CD154 reach their peak expression only after 6 hours of stimulation, therefore both of these antibodies should be incubated with the cells throughout the stimulation time period. The best time to stimulate IL-10 and TGF is between 12 and 24 hours, and serum-free media is better for detecting TGF [11-13].

Result and Discussion

We first investigated the pattern of cytokine staining in established 1 and 2 clones, known only to produce each cytokine by reverse transcription PCR, in order to validate the method of cytokine analysis by flow cytometry. IFN- γ and IFN- β are mutually exclusive and exhibit different kinetics during intracellular synthesis in established 1 and 2 clones. This study's objective was to simultaneously analyze

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