Journal of Cytokine Biology

A Brief Review Intracellular Cytokine Labeling of T helper Cell in Cytokine Function

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Abstract

Based on their cytokine profile, CD4 + T helper (Th) cells can be divided into several kinds. Th1 and Th2 cells are characterised by these polarised patterns of cytokine production, and they may be separated from one another functionally by producing IFN- and IL-4, respectively. The kind of immune response that arises following antigen priming depends on these characteristics. They lack distinguishing surface indicators, and single-cell characterisation by cytokine immunoassay or mRNA analysis is constrained in both cases. We have investigated the formation of Thl and Th2 cells in response to antigen exposure and the patterns of cytokine generation in established T cell clones using immunofluorescent detection of intracellular IFN-/and IL-4. By 4 hours, almost all cells had IFN- production that could be detected, and the majority of cells continued to produce IFN- for >24 hours. At 4 hours, Th2 cell IL-4 output peaked before falling down quickly. Fewer cells generated IFN-% in Th0 cells that contained both cytokines; this phenomenon did not emerge until the production of IL-4 decreased. Clone co-cultivation failed to reveal any such cross-regulation. Th2 cells were produced after antigen-stimulated transgenic T cells expressed an ovalbumin-specific T cell receptor, most likely as a result of natural IL-4 synthesis. Thl cells appeared when IL-12 and/or anti-IL-4 were added, although some Th0 cells appeared when IL-12 was administered alone.

Keywords: Cytokine profile; Immunofluorescent; Acute lymphoblastic leukaemia; Fluorochromes

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]. C D4 Th cells can be divided into various categories that each release distinctive clusters of cytokines, some of which regulate cell-mediated immune responses and others of which promote B cell synthesis of antibodies. Th2 cells produce additional cytokines, such as IL-4, IL-5, IL-10, and IL-13, which guide allergic or anti-inflammatory responses as well as support some B cell responses. Thl cells produce IL-2, IFN-', and lymphotoxin, which enhance delayed-type hypersensitivity responses. In murine and human systems, Th cells that produce cytokines typical of both Thl and Th2 clones have also been reported. These cells are known as Th0 cells, and they may be the polarised Thl and Th2 phenotypes' ancestors [2]. In other circumstances, however, Th0 cells might indeed be a distinct, stable population. Interest in cytokines as treatments is growing. To explore the intrinsic biological features of natural ligands, therapeutic cytokine discovery is typically restricted to tissue targeting, affinity maturation, and/or half-life extension. More recently, cytokine engineering efforts have shown that protein design and selective structure-based engineering can reduce cytokine pleiotropy [3]. However, unlike multi-pass transmembrane proteins that respond to small molecule agonists, such as GPCRs and ion channels, cytokines are proteins that signal through type-I single-pass transmembrane receptors, which pose significant difficulties for the discovery of small molecule agonists. The mLN is where early SFB-induced Th17 cell differentiation takes place. Early RORt expression by SFB-specific Th17 cells requires IL-6. IL-6, IL-21, and IL-23 play various roles in the generation of Th17 effector cytokines, and IL-21 or IL-23 induce delayed differentiation of Th17 cells in the absence of IL-6 [4].

Material and Method

T cells are activated to produce responses by being exposed to liquid stimulants in tubes, plates, or lyophilized stimulants in plates. To improve cytokine responses, the costimulatory antibodies CD28 and CD49d are occasionally introduced [5]. During stimulation, a Golgi-blocking drug (such as monensin or brefeldin A) is also added to maintain the produced cytokine inside the cell. Depending on the markers to be assessed, a different blocking agent and stimulation time will be used. If possible, a control sample that was not stimulated but was subjected to the same circumstances is supplied for comparison [6].

Fluorophores and antibodies

It might be difficult to decide which antibodies to use with which fluorochromes, especially when using multiparameter flow cytometry. The quantity and type of lasers in the apparatus will undoubtedly restrict the range of fluorochromes that can be used. The brightest fluorochromes among them must typically be saved for the faintest markers, when the greatest resolution sensitivity is needed. This enables the use of brightly staining agents like CD3, CD4, or CD8 with weaker fluorochromes [5,6]. Avoiding spillover from bright populations into detectors that would be most negatively impacted by overlap problems, such as those with dim markers or with extremely uncommon occurrences, is especially crucial when creating an antibody cocktail. For instance, one should avoid combining a strongly staining AmCyan

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 workflows [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 Th1 and Th2 clones, known only to produce each cytokine by reverse transcription PCR, in order to validate the method of cytokine analysis by flow cytometry. IFN-/ and IFN-/ are mutually exclusive and exhibit different kinetics during intracellular synthesis in established Th 1 and Th2 clones. This study's objective was to simultaneously analyse intracellular synthesis of IFN-/ or IL-4 by maturing T cells derived from naive TCR-etl3 transgenic CD4+ T cells challenged with particular antigen in the presence of IL-4 or IL-12 using flow cytometry. It is unclear, in example, whether cells within a Th1 or Th2 population primarily generate IFN- and IL-4 or whether they are able to create both cytokines at the same time. We also address this in Th0 clones that have been shown using immunoassay to produce both cytokines. The platform allows for quick, label-free metabolic measurements from a small number of cells, allowing for the early detection of spectral markers and metabolic pathways associated with T-cell activation and differentiation. As opposed to traditional techniques of T-cell evaluation, this is accomplished while using less analytical time and resources.

Conclusion

This work highlights the potential of in-process monitoring and dynamic feedback control strategies via metabolic modulation to drive T-cell activation, proliferation, and differentiation throughout biomanufacturing, in addition to opportunities for fundamental insights into the dynamics of T-cell processes. Brefeldin A has the largest impact on IL-2, IL-4, IL-5, IL-13, IFN, and MIP-1. With monensin, CD107, CD154, IL-10, and TGF all perform better. Finally, monensin has no impact whatsoever on TNF secretion.

Acknowledgement

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