Cytokine 46 (2009) 1–6

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.elsevier.com/locate/issn/10434666

Review Article

Signals for the execution of Th2 effector function

Deborah J. Fowell *

David H. Smith Center for Vaccine Biology and Immunology, Aab Institute of Biomedical Sciences, Department of Microbiology and Immunology, University of Rochester, 601 Elmwood Avenue, Box 609, Rochester, NY 14642, USA

ARTICLE INFO

Article history:
Received 13 November 2008
Received in revised form 10 December 2008
Accepted 29 December 2008

Keywords:
Th2
IL-4
Signaling
Effector function
Inflammation

ABSTRACT

Appropriate control of infection depends on the generation of lymphocytes armed with a particular array of cytokine and chemokine effector molecules. The differentiation of naïve T cells into functionally distinct effector subsets is regulated by signals from the T cell receptor (TCR) and cytokine receptors. Using gene knock-out approaches, the initiation of discrete effector programs appears differentially sensitive to the loss of individual TCR signaling components; likely due to differences in the transcription factors needed to activate individual cytokine genes. Less well understood however, are the signal requirements for the execution of effector function. With a focus on Th2 cells and the kinase ITK, we review recent observations that point to differences between the signals needed for the initiation and implementation of cytokine programs in CD4+ T cells. Indeed, Th2 effector cells signal differently from both their naïve counterparts and from Th1 effectors suggesting they may transduce activation signals differently or may be selectively receptive to different activation signals. Potential regulation points for effector function lie at the level of transcription and translation of cytokine genes. We also discuss how provision of these execution signals may be spatially segregated in vivo occurring at tissue sites of inflammation and subject to modulation by the pathogen itself.

1. Introduction

The control of microbial infection by CD4+ T cells depends on the acquisition and delivery of appropriate effector function to the infection site. Gain of function, the ability to produce a restricted set of effector molecules such as cytokines and chemokines, is attained upon initial T cell activation and differentiation in the lymph node draining the site of infection. Once armed, the effector cells home to the site of infection guided by chemokine and adhesion cues and require re-activation at the infection site to exert their anti-microbial functions. Some of those effector cells will receive additional signals (as yet ill-defined) that support long term survival as functional memory cells. Since the initial description of functionally distinct CD4+ T cell subsets (Th1 and Th2) by Mosmann and Coffman in the 1980's, we have gained enormous molecular insight into the signaling and transcriptional regulation that controls the initial differentiation of naïve CD4+ T cells into distinct subsets: Th1, Th2, Th17 and induced regulatory T cells (iTreg) [1]. However, the signals that the effector T cells require for the synthesis and secretion of effector molecules at the infected tissue site are poorly understood. Nonetheless, a number of studies highlight that the signaling requirements for expression of a given cytokine gene differ in naïve and effector/memory T cells [2–4]. A better understanding of the regulation of effector T cell function should help in the design of therapeutic strategies to promote or suppress immune function at peripheral sites of inflammation.

2. Rapid cytokine production by effectors

The hallmark of effector and memory cells is their ability to rapidly express and secrete high levels of effector cytokines in response to antigen stimulation. High-level cytokine production appears critical for effective immune function. Indeed, in a detailed study by the Seder group designed to define the immune criteria for effective vaccine strategies, the amount of cytokine produced by individual effector cells positively correlated with vaccine efficacy [5]. The mechanisms that facilitate this heightened response are poorly defined. Production of Th2 cytokines at high levels has been linked to chromatin remodeling at three conserved noncoding sequences in the IL-4 locus: the CNS-1 region, the inducible DNase I hypersensitivity (DHS) site V_A and adjacent CNS-2 region and the conserved intron 1 sequence of IL-4 (CIRE) [6–8]. Deletion of the CNS-1 regulatory region in mice led to a modest (2 to 4-fold) reduction in IL-4, IL-5 and IL-13 production by Th2 cells (but not in mast cells) that compromised the ability of mice to mount Th2 responses to a variety of infections, implicating a general role for the region in transcription of cytokines in the IL-4 locus in CD4+ T cells [6]. The inducible 3′ enhancer (DHS V_A) is essential for high-level IL-4 production in both 'mature' Th2 cells and mast cells [7]. This

* Fax: +1 585 273 2452.
E-mail address: Deborah_Fowell@urmc.rochester.edu

1043-4666/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.cyto.2008.12.023

region binds both GATA3 and NFAT1 suggesting TCR signals that fail to support the activation of these transcription factors will strongly impact the amount of IL-4 produced by Th2 effectors. Indeed, continued expression of GATA3 in Th2 effectors is critical for high-level Th2 cytokine production revealed by a number of conditional deletion approaches [9–11]. GATA3 does not appear to be essential for the enforcement of the remodeled chromatin structure in Th2 cells but plays an important role in the transcription of Th2 cytokine genes, possibly through binding to the inducible 3′ enhancer and the intronic regulatory element CIRE for IL-4 and the promoter region of IL-5 and IL-13 [12]. In contrast, the *Mixed-Lineage Leukemia* (MLL) gene, a mammalian homologue of the *Drosophila trithorax* a epigenetic transcriptional regulator, has been implicated in function of Th2 memory cells at the chromatin level [13]. MLL was not required for the induction of the Th2 lineage or IL-4 production in effector cells but was required for maintaining histone modifications at the GATA3 and Th2 locus necessary for optimal IL-4 production in memory Th2 cells. In addition, a recent study shows that memory T cells maintain higher levels of NFAT protein expression than do naïve cells [2], suggesting that resting memory T cells may be poised for transcription by virtue of an enhanced pool of available transcription factors. Thus a combination of greater accessibility for transcription of specific cytokine genes and the increased expression of transcription factors such as NFAT may assist in the rapid production of cytokines by effector T cells.

3. Distinct biochemical responses to TCR engagement in Th2 effectors

During the differentiation process from naïve to effector, Th1 and Th2 cells begin to utilize different TCR-driven signaling components (Fig. 1). Unlike Th1 cells, Th2 cells loose the ability to induce a high and sustained calcium flux and have reduced TCR-triggered tyrosine

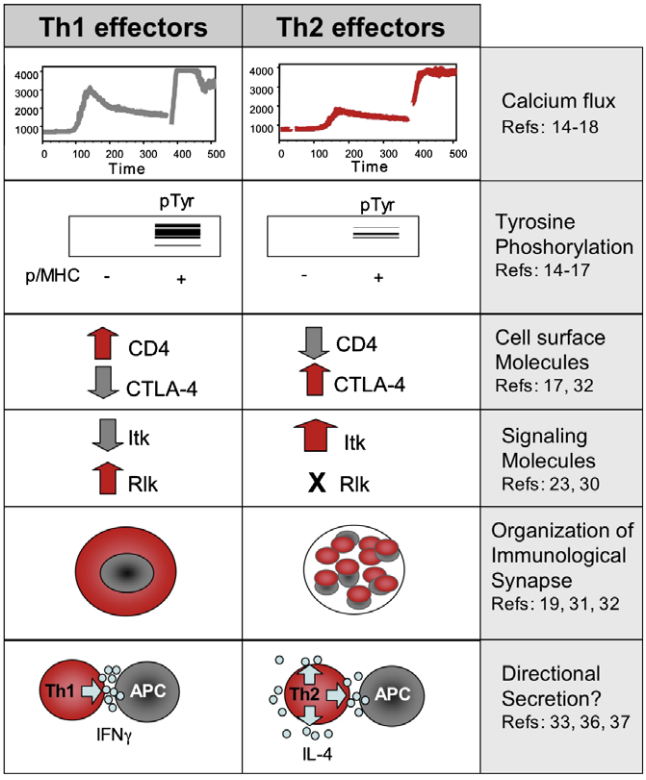

Fig. 1. Signaling differences in Th1 and Th2 effectors.

phosphorylation [14–17]. Both a difference in Ca^{2+} clearance from the cytosol and smaller Ca^{2+}-activated K^+ currents contribute to the lower Ca^{2+} response in Th2 cells [18]. Poor proximal signaling in Th2 cells can partly be explained by a decrease in the expression of CD4 on the cell surface. Th2 cells have been found to express 2-fold less CD4 on their cell surface than Th1 cells [17] and to poorly recruit CD4 to lipid rafts on TCR ligation [19]. The functional significance of this reduction in CD4 was revealed by restoration of CD4 expression levels in Th2 cells using retroviral transfer [17]. Th2 cells expressing CD4 levels comparable to that of Th1 cells showed more robust protein tyrosine phosphorylation and elevated Ca^{2+} signaling. The rationale for decreased CD4 expression in Th2 cells remains to be determined. Interestingly, targeted deletion of CD4 renders CD4 cells unable to differentiate into Th2 cells but leaves Th1 responses intact [20]. Therefore while Th2 cells express less CD4 on their surface they appear more dependent on that CD4 pool for effector function than Th1 cells.

Expression of distinct TEC-family kinase members in T effectors provides an additional level of differential control of TCR signaling. The TEC-family kinases are important amplifiers of the calcium flux through activation of PLCγ, amongst other functions [21,22]. Naïve CD4+ T cells express predominantly ITK and to a lesser extent RLK and TEC. On T cell activation RLK expression is downregulated and is only re-expressed in Th1 effectors. During Th2 differentiation, loss of RLK is accompanied by an increase in ITK expression [23]. The resulting effector cells express ITK if Th2 and RLK and ITK if Th1. Indeed, Th2 cells are heavily dependent on ITK for the calcium flux: ITK-deficient Th2 cells have severely compromised calcium fluxes that correlate with the abrogation of Th2, but not Th1, effector function [23–26]. In addition, RLK appears to directly regulate IFNγ cytokine gene expression by translocation to the nucleus and DNA-binding to a region upstream of the IFNγ transcriptional start-site [27,28]. Although a specific role for RLK in Th1 function remains unclear; given RLK-deficient mice show little attenuation of Th1-dependent immune response [29] and ectopic expression of RLK in ITK-deficient cells can rescue some Th2 effector function [30]. Nevertheless, during differentiation the expression levels of signal components are re-set and accompany specific effector functions on subsequent re-stimulation.

Differences in the signal transduction patterns between Th1 and Th2 cells may partly be the result of altered immunological synapse formation. A number of studies have observed a difference between Th1 and Th2 cells in the organization of molecules at the interface between the effector T cell and the antigen presenting cell. The T cell receptor and CD4 were efficiently recruited to lipid rafts in Th1 cells but not Th2 cells [19] and correlated with a decrease ability of Th2 cells to respond to low-affinity peptide stimulation. The synapse structure in Th2 cells differs from naïve and Th1 cells in the distribution of a number of signaling molecules including TCR, PKCθ, CD45 and talin. In general, the synapse in Th2 cells is less organized with a failure to form the classic 'bulls-eye' patterning of central TCR and peripheral ICAM/LFA-1/Talin seen in non-polarized CD4 T cells and Th1 effectors [31]. Interestingly, these differences may stem from expression of higher levels of CTLA-4 in Th2 cells [32]. Manipulation of CTLA-4 expression levels correlated with altered synapse organization: CTLA-4-deficient Th2 cells were able to form ordered synapses with central TCR clustering while reintroduction of CTLA-4 disrupted TCR clustering [32]. The functional outcome of these experimental changes in Th2 synapse structure with respect to effector cytokine production were not explored; thus the linkage between synapse structure, downstream signaling and cytokine production remains obscure.

An interesting alternative explanation for differences in synapse structure in Th1 and Th2 cells is the role of the synapse in delivery