Cytokine 51 (2010) 227–231

Contents lists available at ScienceDirect

Cytokine

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

Review Article

Cytokine dependent and independent iNKT cell activation

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ARTICLE INFO

Article history:
Received 12 April 2010
Received in revised form 29 April 2010
Accepted 30 April 2010

Keywords:
Natural killer T cells
CD1d
Cytokines

ABSTRACT

Invariant NKT (iNKT) cells have been extensively studied throughout the last decade due to their ability to polarize and amplify the downstream immune response. Only recently however, have the various mechanisms underlying NKT cell activation begun to unfold. iNKT cells have the ability to respond as innate immune cells with minimal TCR involvement as well as through direct TCR recognition of glycolipid antigens. Additionally, the existence of several subsets of iNKT cells creates the potential for other unique pathways, which are not yet clearly defined. Here, we provide an overview of the known mechanisms of invariant NKT cell activation, focusing on cytokine driven pathways and the resulting cytokine responses.

1. Introduction

NKT cells comprise a distinct subset of immune cells expressing both a T cell receptor as well as classical NK cell markers. NKT cells have been categorized into four different groups [1]. The most studied category, consisting of type 1 NKT cells (or invariant NKT cells) is well conserved in mammals and expresses a TCR comprised of an invariant Vα14–Jα18 TCR (human Vα24–Jα18) α-chain paired with a limited subset of TCR Vβ-chains [1]. iNKT cells respond primarily to lipid antigens presented by the non-classical MHC molecule, CD1d. iNKT cells have been described as innate immune effector cells because they are capable of rapidly responding to antigen, releasing cytokines, proliferating and producing cytolytic mediators [2–5]. Their ability to respond quickly to antigen challenge is believed to bridge the gap between the fast acting less specific innate immune system and the antigen specific adaptive response. Significant progress in understanding the function of iNKT cells has relied on α-galactosylceramide (α-GalCer), a strong iNKT cell agonist [6,7]. In addition, critical tools such as CD1d tetramer [8,9], CD1d deficient mice [10–12] and Jα18 deficient mice [6] have been instrumental in promoting recent progress in the CD1d/iNKT cell field. Using these tools and a variety of experimental strategies, researchers have been actively pursuing the identification of the self-antigen that is presented by CD1d. Another active area of research is determining the role of iNKT cells during infection. Several bacterial glycolipid ligands have been shown to activate iNKT cells, supporting a critical role for iNKT cells in some infections [13–16]. Interestingly, even in the absence of specific Ag, iNKT cells have been shown to contribute directly or indirectly to the immune response to several pathogens.

In this review, we summarize the different mechanisms of iNKT cell activation. We also discuss how these different pathways influence the cytokines secreted by iNKT cells.

2. TCR mediated activation

iNKT cells are unique T cells that can respond within minutes to specific Ag. Classical activation of iNKT cells is mediated through TCR recognition of a CD1d presented glycolipid (Fig. 1). Most of the initial investigations used the strong and prototypical agonist α-GalCer [3,5]. Using CD1d deficient mice, it was demonstrated unequivocally that CD1d is critical for the presentation of α-GalCer to iNKT cells. Many studies subsequently utilized α-GalCer to determine the specific requirements needed for optimal iNKT cell activation. Additionally, the roles of several cytokines essential for activation of classical T cells such as IL-12, IL-18 and IFN-α were investigated. Due to the fact that downstream NK cell activation is dependent on these cytokines, there was initially some confusion regarding their roles in stimulating NKT cells. Most of the *in vivo* and *in vitro* data clearly indicate however that initial iNKT cell activation is mostly independent of these cytokines. Essential tools such as CD1d tetramer allowed investigators to discriminate between iNKT cells and NK cells to specifically demonstrate that inflammatory cytokines as well as co-stimulatory molecules were dispensable in this context [17,18]. Other investigators using soluble CD1d coated on a plate showed *in vitro* that agonist glycolipids

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doi:10.1016/j.cyto.2010.04.016

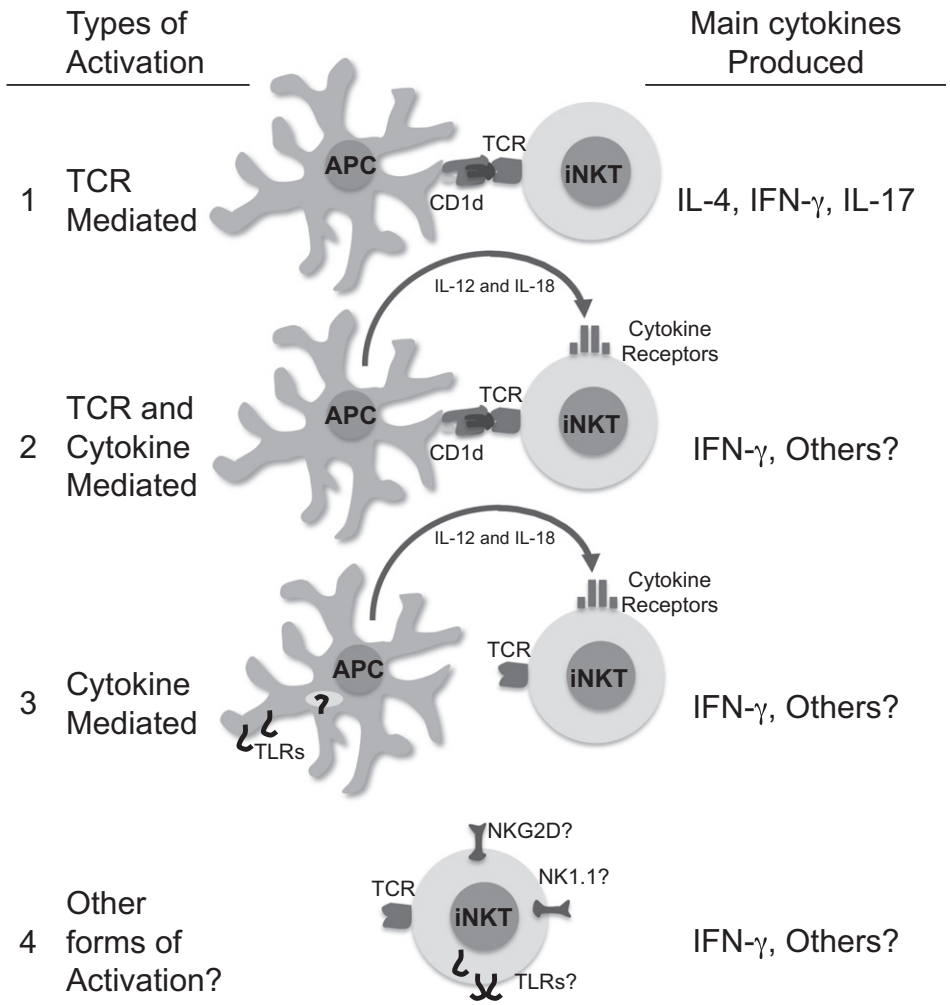

Fig. 1. iNKT cell activation pathways.

bound on CD1d were sufficient to activate iNKT cells [3]. These findings were physiologically confirmed with several recently identified microbial glycolipids from α-proteobacteria, such as *Sphingomonas*, *Ehrlichia*, *Rickettsia*, as well as *Borrelia*. These agonist glycolipids can directly activate iNKT cells [13–16] independently of TLR or IL-12. Similarly to α-GalCer, the *Sphingomonas* glycolipids have been shown to induce the release of IFN-γ and IL-4 [13]. However, it has been shown that dendritic cells pulsed with α-GalCer or *Sphingomonas* glycolipids preferentially stimulate the production of IFN-γ rather than IL-4 [13,19].

This and other findings led to the development of glycolipid analogs and strategies to polarize iNKT responses [20–23]. Polarization of the iNKT cell response has been observed using α-GalCer analogs, however the mechanism leading to this polarization is still under intense investigation [24–26]. Regardless of the mechanism(s), it was recently shown that although T_H1 and T_H2 α-GalCer analogs can induce the predicted systemic cytokine bias, the immediate iNKT cell response is not polarized [27]. Importantly, direct iNKT cell activation with analogs is not without consequences, as overstimulation of iNKT cells can result in iNKT-cell anergy [28,29].

2.1. Subset of iNKT cells activated during this pathway

iNKT cells have been subdivided into several subsets based on cell surface marker expression. Analysis of these subsets demonstrated that they do not respond identically to a stimulus. For instance when stimulated by α-GalCer, human $CD4^+$ iNKT cells produce both T_H1 and T_H2 cytokines, whereas $CD4^-$ (mostly double negative) iNKT cells produce mainly T_H1 cytokines [30,31]. Notably, this dichotomy has not been observed in the mouse. Whether this difference in cytokine production exists in response to α-proteobacteria derived iNKT cell agonists has yet to be determined.

Other subsets of iNKT cells have been recently characterized. These include a distinct IL-17-producing cell subset [32,33], termed iNKT17 cells, as well as a subset that expresses the IL-25R [34]. iNKT17 cells differ from their classical counterpart by lack of NK1.1 expression, presence of ROR-γt which is essential for their alternative developmental program, and their prevalence in the peripheral lymph nodes [35]. NK1.1 negative iNKT cells produce IL-17 upon recognition of exogenous glycolipids derived from *Sphingomonas wittichii* and *Borrelia burgdorferi* [33] as well as by direct cross-link of CD3 and CD28 [36], suggesting that engagement of the invariant TCR is sufficient to stimulate iNKT17 cells.

3. TCR and cytokine mediated activation

Bacteria such as *Salmonella typhimurium*, *Staphylococcus aureus* and *Mycobacterium tuberculosis* that lack agonist glycolipids have been reported to activate iNKT cells through recognition of endogenous lysosomal glycosphingolipids, presented by pathogen-activated dendritic cells [14,37–39]. The identification of the CD1d