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Review

NK T Cell-NK Cell Cross-Talk: Reciprocal Interaction and Activation?

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Abstract. Initiation and propagation of the immune response is the result of a series of coordinated cellular and biochemical interactions that lead to the activation of multiple cell types. It is now clear that an optimal immune response requires precise and rapid communication between different cell subsets. This phenomenon, referred to as cross-talk, is believed to be an essential component of the immune response that provides necessary inflammatory mediators and cytolytic activity for controlling infections and diseases. An example of effective cooperation between different cell types has been recently illustrated by the finding that specific activation of CD1-restricted natural killer T cells (NK T) can quickly lead to the activation of other subsets of cells, such as natural killer (NK) and CD8 T cells.

Key words: T cells cross-talk; NK cells; NK T cells; NK activation.

Introduction

Natural killer T cells (NK T) have recently aroused interest in part because of their ability to secrete both T helper (Th)1 and Th2 cytokines and also because of their ability to elicit a downstream activation of natural killer (NK) cells, CD8 T cells, B cells and dendritic cells (DCs) rapidly[9, 10, 13, 21]. The recruitment of cells from both the innate arm (NK cells and DCs) and the adaptive arm (T cells) of the immune response suggests a versatile function of the NK T cells and may explain some of their adjuvant or regulatory properties. Although there is evidence that interferon γ (IFN-γ) may play a role[10, 13], the molecular mechanism leading to the cross-talk between activated NK T cells and other subsets of cells is not completely understood.

In this review, we will provide an introduction to the three cell subsets involved, specifically NK cells, NK T cells, and DCs, and describe the current model of cooperation between these cell subsets following specific activation of the NK T cells.

Natural Killer Cells

NK cells are dependent upon the bone marrow for development and are present as mature populations in the blood, spleen and liver[34]. They express a variety of cell surface markers that include CD56 in the human and NK1.1 or DX5 in the mouse[24]. Extensive work has been done to define the responses and functions of NK cells during a variety of viral infections[6]. Although

Abbreviations used: NK – natural killer, NK T – natural killer T cell, α-GalCer – α-galactosylceramide, DCs – dendritic cells, B6 – C57BL/6 mice.

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much remains to be learned, the mechanisms contributing to their activation and functions under these conditions are being elucidated at the molecular level[31]. These lymphocytes have the ability to recognize and specifically lyse other cells that have reduced or altered MHC class I expression on their surfaces and are recognized as "non-self"[27, 28]. This is of particular importance in a number of viral infections in which virally induced down-regulation of MHC class I has occurred, preventing $CD8^+$ T cell recognition.

NK cell functions have been shown to be controlled by a group of inhibiting and activating receptors which are specific for a variety MHC class I alleles allowing recognition of infected cells and preventing inappropriate lysis of normal cells[28]. Upon ligand binding, NK cell receptors that contain immunoreceptor tyrosine-based inhibitory motifs (ITIMs) in their cytoplasmic domain prevent NK cell effector function. In contrast, NK cell receptors lacking ITIMs have been implicated in NK cell activation. Some of these activating receptors associate with immunoreceptor tyrosine-based activating motif, bearing adaptor molecules such as DAP12 or DAP10[25]. New evidence suggests that the delivery of positive signals through NK cell receptors contributes to antiviral defense. For example, it was recently shown that the activating receptor Ly-49H[7] directly interacts with a murine cytomegalovirus (MCMV)-encoded protein, m157, expressed on the surface of infected cells[1, 38]. Engagement of Ly-49H with an anti-Ly-49H monoclonal antibody[37] or its viral ligand[1, 38] activates NK cells, initiating both cytokine production and NK cell-mediated cytotoxicity.

NK cells are rapidly recruited to sites of infection and contribute significantly to innate immune responses via robust cytokine production, proliferation, release of cytolytic granules and production of chemokines following activation[35]. Exposure to type I interferons or DC- or macrophage-derived interleukin 12 (IL-12) results in a significant increase of 20–100-fold in the cytolytic activity of NK cells[32]. Substantial production of IFN-γ is induced following exposure to IL-12. NK cell-derived IFN-γ has been shown to be crucial in controlling infections, particularly those involving DNA and RNA viruses such MCMV and vaccinia, respectively, prior to the induction of adaptive immunity[6]. Depletion of NK cells, IFN-γ or IL-12 in murine models of viral infection results in enhanced susceptibility, increased viral titers and a significant decrease in survival[6].

NK cells have also been implicated in tumor surveillance and tumor cell lysis. They have been shown to be cytolytic against a variety of tumor cell lines as well as *in vivo* using multiple murine cancer models[39]. Their cytolytic activity is independent of IL-12 but dependent of perforin, particularly in situations where the tumor is MHC class I deficient. It has also been indicated that these cells may require cell-to-cell contact with DCs to be activated and to initiate innate anti-tumor activities[14, 15].

Natural Killer T Cells

NK T cells are a unique subset of T lymphocytes[4]. These lymphocytes are thymus derived but depend on bone marrow-derived cells for selection[4]. In mice, they are found in the spleen, bone marrow, thymus, and the liver at a concentration of approximately 10^6 cells/organ[23]. In addition to activated T cell markers, such as CD69 and CD44, they also express NK cell markers such as NK1.1 and member of the Ly-49 family. There are four subsets of NK T cells that have been identified to date[23]. However, this review is only concerned with the major NK T cell subset, referred to as Vα14*i* T cells. These lymphocytes have a restricted T cell receptor repertoire that is characterized by expression of a Vα14Jα18 rearrangement (Vα24JαQ in humans) with an invariant junction, preferentially associated with specific Vβ8 chains. Vα14*i* T cells are $CD4^+CD8^-$ or double negative and are dependent on a non-classical, non-polymorphic MHC class I-like molecule, CD1d, for selection, antigen presentation, and activation[33].

Though the identity of the natural ligand(s) of Vα14*i* T cells remains elusive, a synthetic glycolipid, α-galactosylceramide (α-GalCer), discovered by the Kirin Pharmaceutical Research Corporation, has been shown to specifically activate Vα14*i* T cells[8, 19, 22]. TCR cross-linking by CD1d-presented α-GalCer induces rapid cytokine production and results in the disappearance of Vα14*i* T cells[12]. However, it is not clear if this disappearance reflects apoptosis induced cell death or a down-regulation of surface markers that render them undetectable by flow cytometry. Following α-GalCer stimulation, Vα14*i* T cells rapidly produce (within 1 h) large amounts of both Th1 and Th2 cytokines, specifically, IFN-γ and IL-4[4]. This innate immune type of response is unusual for T cells and infers that these lymphocytes are immunoregulatory cells with the potential to direct the immune response very early in its initiation. In fact, with the discovery of α-GalCer and the subsequent development of CD1d tetramers[5, 29], the amount of evidence supporting this idea has increased and numerous studies have indicated that Vα14*i* T cells may play a role in autoimmunity, the

induction of tolerance, tumor surveillance, and response to pathogens[16, 23].

Dendritic Cells

DCs are a heterogeneous population of specialized leukocytes that have the ability to process and present antigens to a variety of lymphocytes, both of the innate and adaptive systems[2, 36]. DCs were initially classified in two major categories, lymphoid DCs and myeloid DCs. However, it is now clear that DCs can be classified in many more subtypes depending on their progenitors, tissue localization and markers expressed on their cell surface[36]. DCs found in non-lymphoid tissues are typically classified as immature DCs. At this stage, they are extremely efficient at antigen uptake and processing but have very low, or absent, surface expression of MHC class II molecules[3, 17, 30]. Therefore, immature DCs must undergo maturation to be effective antigen--presenting cells[3].

Following an encounter with microbial products or cellular debris resulting from necrosis, immature DCs migrate to lymphoid tissues and undergo maturation. Mature DCs lose the ability to take up and process antigens. They upregulate expression of surface MHC class II molecules and additional co-stimulatory molecules and transiently produce substantial amounts of cytokines, particularly IL-12 and type I interferons. Once fully matured, DCs have the ability to induce lymphocyte activation, anergy or apoptosis, providing both a positive and a negative feedback loop for immunoregulation[26].

Studies have shown that DCs also play an integral role in the induction and maintenance of self-tolerance[41]. It is believed that some immature DCs migrate to lymphoid tissues and undergo maturation but do not become fully activated. Instead, they become quiescent, maintaining antigen uptake and processing ability while upregulating expression of moderate levels of MHC class II molecules on their surface. This allows continuous maintenance of tolerance via presentation of self--antigens. Additionally, DCs have the ability to transport antigens from the endocytic compartments to surface MHC class I molecules to cross-prime or cross-tolerize $CD8^+$ T cells, supporting the idea of DCs in the induction of immunity and tolerance[11, 42].

It is well established that DCs are a major source of the Th1-promoting cytokine IL-12, which has been shown to be required for the induction of IFN-γ during the innate immune response and for strong promotion of a Th1-type response[36]. IL-12 production is induced by most pathogens and significantly enhanced by CD40-CD154 interaction between DCs and T cells. In addition to IL-12, DCs have also been shown to produce IL-1β, IL-6, IL-23, IL-18, IFN-α/β and tumor necrosis factor α. The majority of these pro-inflammatory mediators are of vital importance not only in initiation of the response, but also in controlling or clearing infections[36]. DCs are also the source of a variety of chemokines necessary for recruitment of additional immune cells and, consequently, controlling disease. There is substantial evidence that DCs efficiently present α-GalCer *in vitro* and, in turn, produce large amounts of IL-12[20, 43]. Therefore, much like microbial agents, α-GalCer activates DCs through the cognate interaction with CD1d-restricted Vα14*i* T cells.

NK T Cell-NK Cell-DC Cross-Talk: Evidence and Mechanisms

Recent studies have indicated that Vα14*i* T cells can activate NK cells and subsequently induce the activation of T and B cells[9, 10, 13, 21]. Using α-GalCer treatment, it has been observed that NK cells exhibit signs of activation such as IFN-γ production and CD69 induction and begin to proliferate within 90 min after α-GalCer injection[10]. This effect is not seen in Vα14*i* T cell-deficient mice ($CD1d^{-/-}$ or $J\alpha281^{-/-}$ mice), indicating that Vα14*i* T cells are important in the initiation of NK cell activation following α-GalCer treatment[10]. In $RAG^{-/-}$ mice, which lack NK T, T, and B cells but have functional NK cells, treatment with α-GalCer does not result in NK cell activation or proliferation. Therefore, α-GalCer does not directly activate NK cells and the rapid NK cell activation observed is a secondary event, preceded by Vα14*i* T cell activation and IFN-γ production (Fig. 1). These findings have lead to a re-evaluation of the Vα14*i* T cell effector functions against tumors[10]. Indeed, it has been demonstrated that the anti-metastatic effect of α-GalCer was impaired in NK cell-depleted or IFN-γ-deficient mice[18, 40].

It has also been indicated that the cascade of activation initiated by α-GalCer activated Vα14*i* T cells extended to cells of the adaptive system, though delayed in comparison with the rapidity of NK cell activation[9, 10]. The biological mechanism leading to the activation of NK cells, DC and B cells is not yet understood. Clearly, IFN-γ is involved in the cross-talk between Vα14*i* T cells and NK cells, as anti-IFN-γ treatment *in vivo* partially blocked the Vα14*i* T cell-induced activation of the NK cells[10]. It has also been proposed that the Vα14*i* T-induced NK cell activation could be

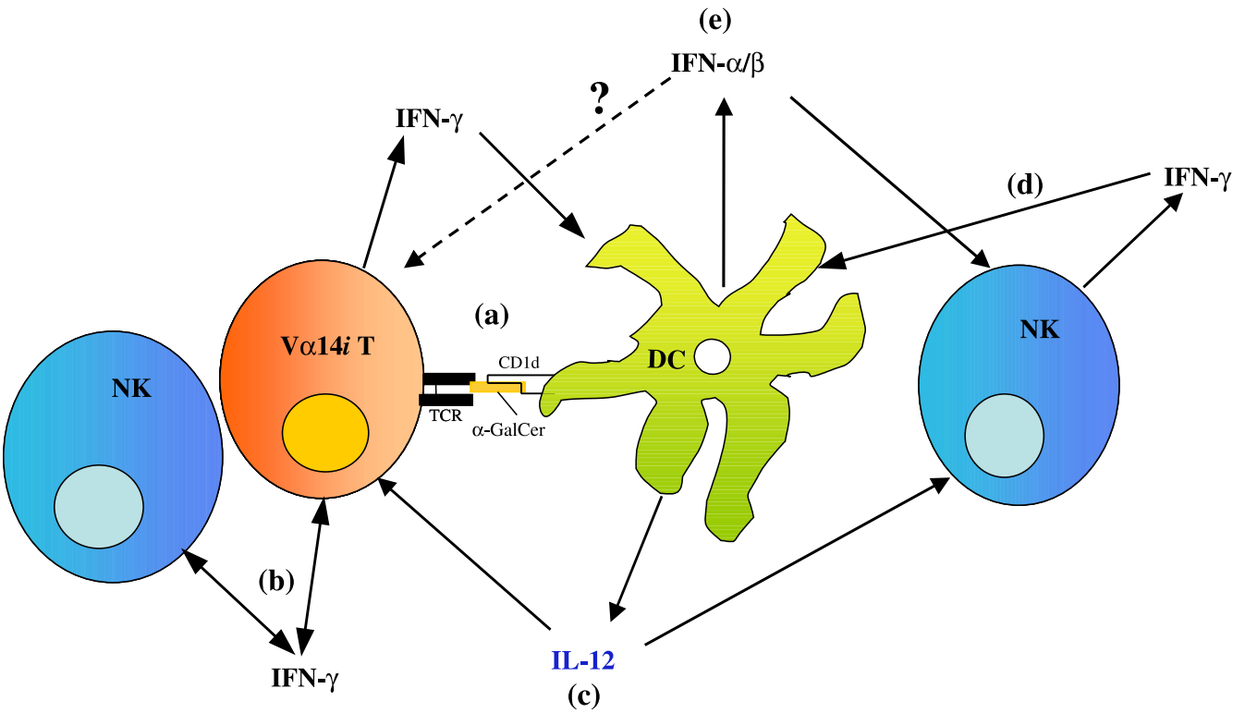

Fig. 1. Current understanding of Vα14*i* T and NK cell cross-talk indicates that, (a) following the presentation of α-GalCer by DCs to Vα14*i* T cells, substantial amounts of IFN-γ and IL-12 are produced by the Vα14*i* T cells and DCs, respectively. The Vα14*i* T cell activation enhances IL-12 production, and possibly IFN-α/β, by DCs and induces NK cell activation and IFN-γ production (b). It is likely that an activation pathway exists in which NK cell-derived IFN-γ precedes and induces Vα14*i* T cell activation and cytokine production, however, this has not yet been determined. It is also uncertain if cell-to-cell contact is required for Vα14*i* T cell activation of NK cells, or vice versa. Though current data do show that (c) NK-derived IFN-γ promotes DC maturation and activation. An additional pathway (d) has also been described in which DC-derived IL-12 induces the activation of Vα14*i* T and NK cells. This pathway could be enhanced by the subsequent production of IFN-γ by either or both Vα14*i* T and NK cells. (e) It has also been indicated that IFN-α/β produced by DCs is important in the induction of NK cell activation and cytotoxicity in viral infections. Therefore, it is possible that type I interferons may also play an important role in the activation and function of Vα14*i* T cells. The events illustrated in this figure are not mutually exclusive

caused by the Vα14*i* T cell-mediated stimulation of DCs to release IL-12 (see model Fig. 1). Indeed, both NK and Vα14*i* T cells express the IL-12 receptor and respond to IL-12. However, neutralization of IFN-γ in IL-12-deficient mice does not completely abolish the cross-talk between Vα14*i* T cells and NK cells[13]. This suggests that factors other than IL-12 and IFN-γ contribute to the cross-talk between Vα14*i* T and NK cells. In addition, the possible activation of Vα14*i* T cells as a consequence of the activation of NK cells has not been demonstrated and will require future examination. The demonstration that a "reverse cross- -talk" exists between NK and Vα14*i* T cells is, however, complicated by the lack of reagents that directly and specifically activate NK cells *in vivo*. Although Vα14*i* T cells are activated during MCMV infection (Wesley et al., unpublished observations), it is not clear whether the Vα14*i* T cell activation is a direct consequence of the NK cell activation or a bystander activation provoked by the DC-derived IL-12 in response to the viral infection.

Concluding Remarks

The discovery of α-GalCer as an antigen for Vα14*i* T cells has been particularly useful in examining their potential functions. Using this antigen, it was found that injection of α-GalCer induced a rapid cascade of cellular activation that begins with the activation of Vα14*i* T cells and propagates rapidly to other innate cells, such as NK cells and DCs, and to adaptive B and T cells. The Vα14*i* T cells activation of NK cell, DCs and $CD8^+$ T cells involves complex biochemical and biological interactions. A better understanding of these interactions will certainly determine the potential of fu-

ture vaccines or adjuvants based on the immune properties of Vα14*i* T cells.

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