achievement of additional insights into signaling events that are mediated by each of the TLRs and their adaptors, and the delineation of non-TLR-mediated pathways with similar effects, seem a certain road to travel to achieve our goal of developing safe and powerful vaccine adjuvants.

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The NKT cell system: bridging innate and acquired immunity

Masaru Taniguchi, Ken-ichiro Seino & Toshinori Nakayama

Although natural killer T cells are activated during infection, it is not clear how this process occurs. Closer examination indicates that recognition of endogenous ligands and interleukin 12, rather than bacterial products, may drive the activation process.

The innate immune system is characterized by rapid responses to pathogens and is mediated mainly by macrophages, dendritic cells, granulocytes and natural killer (NK) cells. In contrast, the acquired immune system, composed of T and B lymphocytes, is characterized by memory or secondary antigen-specific immune responses. Among the cell types that have been postulated to link the two arms of the immune system, CD1d-restricted NKT cells are compelling candidates, being able to respond rapidly and subsequently to activate other cell types[1,2]. However, the detailed mechanism of NKT cell activation is unclear at present. In this issue of *Nature Immunology*, Brigl *et al.* provide evidence for the important functional involvement of self ligands in the activation of NKT cells during infection, and thus add to our understanding of NKT cells as a 'bridging system' between innate and acquired immunity[3].

Because of their apparent self-reactivity and ability to quickly release large quantities of cytokines such as interferon-γ (IFN-γ), NKT cells are hypothesized to be important in the initiation and regulation of various immune responses[1,2]. Accordingly, the function of self ligand in the activation of NKT cells and the function of NKT cells in the immune system have been subjects of much recent research, debate and speculation. Although the invariant $V_\alpha14$ receptor expressed by NKT cells specifically recognizes an unusual glycolipid, α-galactosylceramide (α-GalCer), in conjunction with CD1d[4], α-GalCer is an exogenous ligand. Thus, the presence of endogenous self ligands and their physiological importance continues to be a source of much speculation. This speculation is fueled by the observation that NKT cells seem to be constantly activated *in vivo*; freshly isolated NKT cells express activation markers such as CD69 and CD44. In addition, the use of the TCR $V_\beta8$ chain by NKT cells is restricted to one or two invariant sequences that are distinct in different tissues[1]. Finally, because no NKT cells develop in the absence of CD1d, it seems that developing NKT cells that recognize self ligands presented on CD1d are positively selected[5].

Although the molecular basis of self ligands for NKT cells remains unclear, their self-reactivity seems to constitute an important step in the initiation of protective immunity. The first cells in the innate system to be activated during an infection are dendritic cells. This activation is mediated by Toll-like receptors (TLRs), which sense bacterial products[6], leading to activation of the transcription factor NF-κB and the production of proinflammatory cytokines (interleukin 1 (IL-1), IL-6 and tumor necrosis factor-α), IL-12 and the upregulation of costimulatory molecules (CD80 and CD86) on dendritic cells (**Fig. 1**). Most importantly, IL-12 is essential for the activation of NKT cells and their subsequent production of IFN-γ during infection[3,7]. Indeed, only NKT cells, but not other cells such as naive T cells or NK cells, express substantial amounts of IL-12 receptor components[1], and NKT cells are primary targets for IL-12 for inducing antitumor activity *in vivo*[8,9]. However, although IFN-γ production by NKT cells is crucial for the induction of efficient protective immunity against pathogens, the precise mechanisms and the initial trigger events are unclear.

Brigl *et al.* now provide an answer to this important question[3]. During the first 2 or 3 days after infection, weak responses by NKT cells to self ligands are amplified by dendritic cell–derived IL-12 in response to TLR activation by microbial products, resulting in the production of IFN-γ. Thus, both IL-12-induced signaling and NKT cell activation by self ligands are necessary to initiate pathogen-specific immune responses. However, the recognition of a pathogen-derived cognate antigen is not required for NKT cell activation. Despite the ability of CD1d to present glycolipid antigen and the recognition of this presentation by $V_\alpha14$ receptor, pathogens do not seem to activate NKT cells directly. Instead, microbial products seem to be important only in stimulating dendritic cells through TLR signaling to produce IL-12.

Although recognition of self ligands and the subsequent weak responses of NKT cells are essential at the initial phase of bacterial infection, this self-reactivity does not elicit any effector functions of NKT cells *in vivo*. In addition, in the absence of dendritic cells, IL-12 alone does not activate NKT cells. Thus, the recognition of self ligand–CD1d is required for IL-12-mediated NKT cell activation. In contrast, the recognition of α-GalCer induces signals to activate NKT cells efficiently even in the absence of IL-12,

Masaru Taniguchi, Ken-ichiro Seino & Toshinori Nakayama are in the Laboratory for Immunoregulation, RIKEN Research Center for Allergy and Immunology, Tsurumi, Yokohama and Department of Molecular Immunology, Graduate School of Medicine, Chiba University, Chiba, Japan.
e-mail: taniguti@med.m.chiba-u.ac.jp

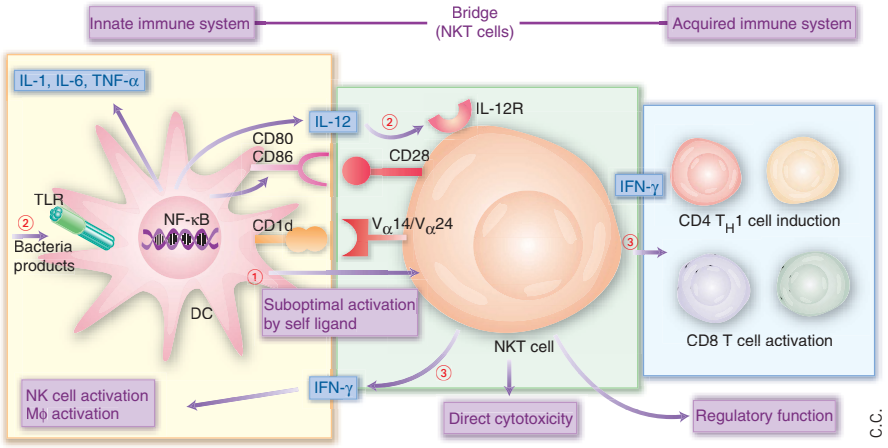

Figure 1 The NKT cell system as a functional bridge between innate and acquired immunity. Both activation of NKT cells by cognate self ligands (1) and IL-12-mediated signaling from dendritic cells induced by pathogens (2) are required for the initial steps of protective immunity. These activation events efficiently induce IFN-γ production from NKT cells (3), which subsequently activates other cell types, such as NK cells and macrophages in the innate immune system as well as CD4 and CD8 T cells in the acquired immune system. IL-12R, IL-12 receptor; T_H1, T helper type 1; DC, dendritic cell; Mϕ, macrophage.

indicating that the molecular mechanism underlying NKT cell activation in physiological conditions is different from those induced by α-GalCer.

This model of NKT cell activation through cognate self ligands amplified by IL-12 has important implications. The mode of NKT cell activation is likely to occur in any microbial challenge that triggers IL-12 production. Furthermore, it might provide some clues for a more general framework for NKT cell activation in a variety of immune responses. The successful identification of α-GalCer and the development of gene-manipulated mice that lack NKT cells ($J_α281$-deficient and CD1d-deficient mice) have helped to elucidate the remarkable functional diversity of NKT cells, such as host defense and immunoregulation[1,5]. These functions include the prevention of tumor development and metastasis; suppression of allergic responses; and protection against viruses, parasites, bacteria and their products. IFN-γ produced by NKT cells also activates pathogen-specific CD4 T helper type 1 responses as well as CD8 T cell–mediated cytotoxic responses against infected targets[7] (**Fig. 1**). These adjuvant or helper effects of NKT cells are directed at pathogen-specific T cells in the acquired immune system, and thus, without the NKT cell system, the elimination of pathogenic microorganisms could be impaired. In addition, IFN-γ produced by NKT cells activates NK cells and macrophages in the innate immune system, thus facilitating inflammatory responses toward pathogens.

Similar mechanisms of NKT cell activation may operate in several other types of immune responses, including tumor immunity. The incidence of methylcholanthrene-induced tumor development is higher in NKT-deficient mice, indicating that NKT cells is crucial in antitumor immune responses[10]. It is possible that NKT cells involved in tumor immunity are activated through the recognition of self ligand–CD1d in the presence of IL-12, rather than directly by tumor products. The primary target cells for IL-12 seem to be NKT cells, as IL-12-induced antitumor effects decrease substantially in the absence of NKT cells[9]. Moreover, both NK cells and conventional T cells fail to produce IFN-γ even after IL-12 activation, and seem to be functionally impaired in the absence of NKT cells[11].

These findings indicate that the NKT cell system works as a functional bridge between the innate and acquired immune systems. However, unlike the acquired immune system, the critical initial event for NKT cell activation may not necessarily be mediated by the recognition of foreign antigens but rather by cognate self ligands and IL-12 receptor–mediated signaling. The manipulation of dendritic cells to produce IL-12 is a promising strategy for vaccine development, as agents that induce IL-12 production could be used to selectively trigger T helper type 1 immune responses through the NKT cell system. Thus, the NKT cell system can be considered as a key target for vaccine development aimed at protection from infectious diseases, augmentation of antitumor responses, and regulation of the onset and progression of various diseases, including immunoglobulin E–mediated allergic disorders.

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