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Review

Adjuvant activity mediated by iNKT cells

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ABSTRACT

Invariant natural killer T (iNKT) cells have adjuvant activity due to their ability to produce large amounts of IFN-γ, which activates other cells in innate and acquired systems, and orchestrates protective immunity. Based on these adjuvant mechanisms, we developed iNKT cell-targeted adjuvant therapy and carried out a phase I/IIa trial on advanced lung cancer patients. The patient group with increased numbers of IFN-γ-producing cells showed prolonged survival with a median survival time of 31.9 months. Sixty percent of the patients in this group survived for more than 2 years with only a primary treatment and without tumor progression and metastasis.

1. Introduction

Invariant natural killer T (iNKT) cells are characterized by the expression of an invariant antigen receptor encoded by Vα14Jα18 in mice and Vα24Jα18 in humans and also by the rapid production of both Th1 and Th2 cytokines after stimulation with their ligands [1–3]. An exogenous glycolipid, α-galactosylceramide (α-GalCer), has been identified as a ligand for mouse iNKTcells and is presented to these T cells by the monomorphic CD1d molecule. Particular CD1d amino acids (Ser76, Arg79, Asp80, Glu83, and Gln150) that are important for binding with either α-GalCer or the iNKTcell receptor are well conserved among species such as mouse, rat, sheep, and human [4–7]. In addition, the first four amino acids (Asp94, Arg95, Gly96, and Ser97) in the Jα18 region of the iNKTcell receptor important for the binding with α-GalCer and the CD1d molecule are also conserved in mice and humans. Thus, α-GalCer identified can be used to activate both human and mouse iNKT cells. Although α-GalCer is an exogenous ligand, the existence of endogenous self-ligands has been speculated based on the observation that iNKT cells appear to be persistently activated *in vivo*; freshly isolated iNKT cells express activation markers such as CD69 and CD44. Moreover, because no iNKT cells develop in the absence of CD1d, it appears that developing iNKT cells recognize self-ligands presented by CD1d and are positively selected.

Because of their apparent self-reactivity and ability to quickly release large quantities of cytokines such as interferon-γ (IFN-γ), iNKT cells have been demonstrated to play important roles in the initiation of protective immune responses. In fact, iNKT cells freshly isolated from tissues express large amounts of mRNA for IFN-γ and IL-4, although their apparent self-reactivity does not elicit any iNKT cell effector functions *in vivo*. However, the recognition of self-ligands and the subsequent weak responses of iNKT cells are essential during the initial phase of protective immunity.

In an immune response against pathogens, one of the first cells to be activated is the dendritic cell (DC) of the innate system. This activation is mediated by Toll-like receptors (TLR) on the DCs, leading to the production of pro-inflammatory cytokines and IL-12 and the up-regulation of co-stimulatory molecules (e.g., CD40, CD80, and CD86). Most importantly, IL-12 has been shown to be essential for the activation of iNKT cells, because only iNKT cells, and not other cells such as naïve T cells or NK cells, express substantial amounts of the mature form of the IL-12 receptor (IL-12R), and iNKT cells have been shown to be the primary targets for IL-12.

Weak responses by iNKT cells to self-ligands are further augmented by IL-12 secreted by DCs in response to TLR activation, resulting in the production of IFN-γ by the iNKT cells. Thus, both inherent self-ligand activation and extrinsic IL-12-induced signaling are necessary to initiate iNKT cell-mediated protective immune responses. Although some pathogen-derived glycolipids can directly activate iNKT cells, in most cases the recognition of pathogen products is not required for iNKT cell activation. Thus, pathogen products only seem to play a role in stimulating DCs through TLR-signaling to produce IL-12.

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IL-12 alone does not activate iNKT cells in the absence of DCs, and the recognition of self-ligands by the invariant NKT cell receptor is required for IL-12-mediated iNKT cell activation. By contrast, α-GalCer recognition induces signals that activate iNKT cells efficiently even in the absence of IL-12. Thus, the molecular mechanism underlying iNKT cell activation under physiological conditions appears to be different from that induced by strong non-self-ligands, such as α-GalCer.

After activation by α-GalCer or pathogens, mouse and human iNKT cells exhibit strong adjuvant effects on protective responses by MHC-dependent and -independent activation of various effector cells *in vitro* and *in vivo* [4,8–11]. IFN-γ produced by activated iNKT cells in turn activates various other effector cells, including DCs, NK cells, and neutrophils in the innate immune system, and CD4 Th1 and CD8 T cells in the acquired immune system, which is characterized by memory and secondary antigen-specific immune responses [1,12,13]. Thus, iNKT cells link the two arms of the immune system, forming a bridge between innate and acquired immunity.

Similar mechanisms may be operative in other protective responses, including rejection of tumor cells and prevention of tumor metastasis. iNKT cells involved in tumor immunity appear to be activated through the recognition of endogenous self-ligands in the presence of IL-12, rather than directly by tumor products. Moreover, in the absence of iNKT cells, unstimulated NK cells and conventional T cells both fail to produce IFN-γ even after direct injection of IL-12 and appear functionally impaired. IFN-γ produced by iNKT cells activates both CD4 T helper cell responses and CD8 T cell-mediated cytotoxic responses against tumor targets and also activates the innate NK cells and neutrophils, thus facilitating inflammatory responses toward tumor targets. Activated NK cells, neutrophils, as well as iNKT cells themselves launch a coordinated cytotoxic attack against the tumor cells.

In general, tumors contain MHC^+ and MHC^- cells. MHC^+ tumor cells are eliminated by CD8 killer T cells, because CD8 T cells can recognize tumor antigen in conjunction with MHC I. On the other hand, MHC negative tumor cells are killed by innate immune cells, such as NK cells, because one type of NK receptors delivers negative signals that inhibit NK cytotoxic activity in the presence of an MHC I molecule, the ligand for this class of NK receptor. Thus, NK cells but not CD8 T cells can kill tumor cells that have lost expression of MHC I on their surface. For optimal therapeutic purposes both MHC^+ and MHC^- target tumor cells should be eliminated at the same time, thus activation of the iNKT cell-dependent cellular cascade is an important strategy for treatment of cancer (Fig. 1). These are adjuvant effects of iNKT cells, and without the iNKT cell system, the protective immune responses against tumors could be impaired. The critical initial event for iNKT cell activation is mediated by the recognition of self-ligands and IL-12 receptor signaling. Thus, the manipulation of DCs to produce IL-12 is a promising strategy for treatment of cancer patients to selectively trigger protective immune responses through the iNKT cell system.

2. iNKT cell-mediated adjuvant effects on innate immunity

DCs in the steady state are immature; they are able to capture antigens, but fail to stimulate T cell immunity (Fig. 1). However, iNKT cells can be activated by immature DCs, which is different from conventional T cell activation by peptide/MHC [14,15]. Thus, in the initial step in the iNKT cell–DC interaction, α-GalCer presented on immature DCs activates iNKT cells to proceed to maturation step of DCs.

Concerning maturation of DCs, both co-stimulatory molecule- and cytokine-mediated signals are involved. A single injection of free α-GalCer into mice induces a burst of IL-4 (at 2 h), IL-12 (at 6 h) and IFN-γ production (at 16–24 h) that is robust enough to be detectable in the serum [16]. Phenotypically, up-regulation of co-stimulatory molecules (CD40, CD80, CD86, and B7-DC) and MHC class II on DCs and CD40L expression on iNKT cells are detectable within 2–6 h after α-GalCer administration [17]. This phenotypic maturation returns to the basal level 72 h later. The iNKT cells are necessary for DC maturation because this process does not occur in Jα18-deficient mice (Fig. 1).

iNKT cell-mediated adjuvant effects on the maturation of DCs have been studied in terms of inflammatory cytokines and CD40–CD40L signaling [17]. With regard to the inflammatory cytokines during the early phase after immunization, TNF-α and IFN-γ are initially secreted and detectable in the serum. When TNF-α and IFN-γ signaling is blocked in immunized mice, the co-stimulatory molecules are not up-regulated on DCs, suggesting a requirement for these cytokines. On the other hand, in α-GalCer-treated $CD40^{-/-}$ and $CD40L^{-/-}$ mice, expression of co-stimulatory molecules (CD80, CD86, and B7-DC) on DCs is up-regulated to the same level as in WT mice. The findings have also been confirmed by Matsuda et al., who showed that IFN-γ production from iNKT cells in $CD40^{-/-}$mice can be detected at 2 h after α-GalCer administration [18]. These findings indicate that CD40 signaling is not crucial for the initial activation of DCs, but instead functions as an augmenting factor.

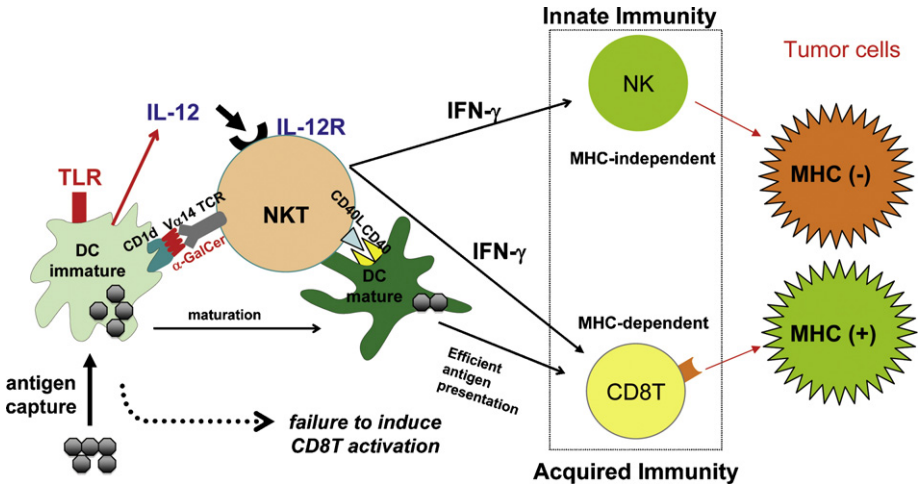

Fig. 1. iNKT cell-mediated adjuvant effects on anti-tumor protective responses. The α-GalCer-loaded immature DCs activate iNKT cells to produce IFN-γ. The resulting augmented expression of CD40L on iNKT cells initiates DC maturation, including the enhanced production of IL-12 and elevated expression of co-stimulatory molecules, which can induce potent antigen-specific $CD4^+$ and $CD8^+$ T cell responses (adaptive immunity). The IFN-γ and IL-12 also induce the activation of NK cells (innate immunity).