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REVIEW

Boosting the immune response: the use of *i*NKT cell ligands as vaccine adjuvants

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Abstract **Natural killer T (NKT) cells comprise a small, but important T cell subset and are thought to bridge the innate and adaptive immune responses. The discovery of NKT cells and extensive research on their activating ligands have paved the way for modulation of these potent immunoregulatory cells in order to improve the outcome of various clinical conditions. Efforts to modulate NKT cell effector functions have ranged from therapy for influenza to anti-tumor immunotherapy. These approaches have also led to the use of NKT cell agonists such as α-Galactosylceramide (α-GalCer) and its analogs as vaccine adjuvants, an approach that is aimed at boosting specific B and T cell responses to a vaccine candidate by concomitant activation of NKT cells. In this review we will provide a comprehensive overview of the efforts made in using α-GalCer and its analogs as vaccine adjuvants. The diverse array of vaccination strategies used, as well as the role of NKT cell activating adjuvants will be discussed, with focus on vaccines against malaria, HIV, influenza and tumor vaccines. Collectively, these studies demonstrate the efficacy of NKT cell-specific agonists as adjuvants and suggest that these compounds warrant serious consideration during the development of vaccination strategies.**

Keywords vaccines, NKT cells and CD1d

Introduction

Natural killer T (NKT) cells were originally described in 1987 by Fowlkes et al. (1987) as murine thymocytes expressing a restricted TCR repertoire. These cells were also found to express NK1.1 similar to Natural Killer (NK) cells and were subsequently named NKT cells (Makino et al., 1995). The majority of NKT cells express an invariant T cell receptor (TCR) α chain rearrangement— Vα14Jα18 in mice, and Vα24Jα18 in humans, associated with Vβ chains of limited diversity, and are referred to as canonical or invariant NKT (*i*NKT) cells (Koseki et al., 1991; Porcelli et al., 1996). In a screen for anti-tumor agents, a glycolipid compound α-Galactosylceramide (α-GalCer, KRN7000) was found to have potent anti-tumor activity (Kobayashi et al., 1995; Natori et al., 1997). This anti-tumor property of α-GalCer was attributed to the specific activation of NKT cells. NKT cells recognize glycolipid antigens in the context of a non-polymorphic major histocompatibility complex (MHC) - like molecule, CD1d (Kawano et al., 1997; Burdin et al., 1998). After the discovery of α-GalCer, many other activating ligands for *i*NKT cells were discovered, all of which share the common characteristic of being presented by CD1d molecules to the *i*NKT cell TCR. Several microbial glycolipids that bind CD1d molecules have recently been described. These include glycosphingolipids from *Sphingomonas* spp., glycolipid antigens from *Borrelia burgdorferi* and diacylglycerols from various pathogenic bacteria (Kinjo et al., 2005; Mattner et al., 2005; Sriram et al., 2005; Kinjo et al., 2006). In addition to these activating ligands, the NKT cell TCR is also known to bind self-glycolipid antigens such as isoglobotrihexosylceramide (iGb3) and glycosylphosphatidylinositol (GPI) (Joyce et al., 1998; Zhou et al., 2004). It is also known that activating ligands are loaded on the CD1d molecule in endocytic compartment s where the CD1d molecules appear after being recycled from the cell surface (Roberts et al., 2002; Zhou et al., 2004). Several groups have also studied the structural aspects of glycolipid antigen

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loading on CD1d molecules using crystal structures of such complexes. These studies have been able to shape a model of 'induced fit', whereby the CD1d molecule, as well as the NKT cell TCR play an active role in determining the ultimate structural conformation of the bound glycolipid (Li et al., 2010b; Yu et al., 2011).

On a more translational track, many attempts have been made to boost immunity through the activation of NKT cells. This area of NKT cell biology is of particular interest since α-GalCer has been shown to enhance specific immune responses elicited by vaccines for infectious diseases, as well as cancer (Yamaguchi et al., 1996; Silk et al., 2004). Many researchers have also been able to synthesize analogs of α-GalCer (Yang et al., 2004; Lu et al., 2006; Blauvelt et al., 2008; Velmourougane et al., 2009; Hogan et al., 2011). The advantage of adopting this strategy is twofold: first, the potency of the ligand can be manipulated by altering its acyl chains and other functional groups. Second, it is possible to streamline the immune response toward a T Helper Type 1 or 2 (Th1 or Th2) bias depending on the condition being treated. *i*NKT cells are known to rapidly produce large amounts of Th1, Th2 and Th17 cytokines (Yoshimoto et al., 1995; Kawakami et al., 2001; Miyamoto et al., 2001; Niemeyer et al., 2008; Yoshiga et al., 2008). This characteristic is thought to be a function of the nature of the activating signal received through the TCR, warranting the search for new analogs of α-GalCer that may be able to skew the response elicited by the primary immunizing vaccine (see schematic in Fig. 1). Activation of NKT cells is also known to rapidly induce NK cell activation, which further boosts the triggered immune responses and modulates the cytokine milieu (Carnaud et al., 1999). Numerous efforts have been directed toward the adjuvantation of vaccines against malaria, Human Immunodeficiency Virus (HIV) and influenza, utilizing NKT cell activating ligands such as α-GalCer and its analogs (see Table 1). In the field of tumor immunology, attempts at using α-GalCer directly as an anti-tumor agent have had some success, but there is now interest in combination therapy for malignancies whereby the activation of *i*NKT cells via α-GalCer can be used as an adjuvant with a tumor-specific antigen (Giaccone et al., 2002). It has also been found that treatment with α-GalCer intravenously leads to the induction of anergy in NKT cells (Parekh et al., 2005). It has been reported that this limitation can be overcome by using α-GalCer as an adjuvant in mucosally administered vaccines since this route of entry ensures a high level of NKT cell activation and avoids the induction of tolerance (Courtney et al., 2011).

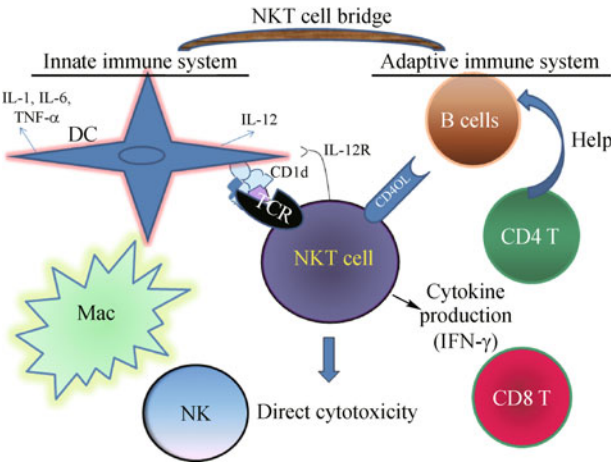

Figure 1 The Role of NKT cell agonists as vaccine adjuvants. NKT cells can bridge innate and adaptive immune responses. NKT cells recognize lipid antigen in the context of CD1d molecules. Following activation, NKT cells rapidly secrete cytokines (i.e., IFN-γ) and can directly mediate cytotoxicity. Cytokine production by NKT cells can lead to the activation of NK cells, macrophages (Mac) and in combination with CD40/CD40L intercitons lead to the maturation of dendritic cells (DC). Thus, the co-administration of an NKT cells agonist,such as alpha galactosylceramide,with a vaccina may be able to enhance the efficacy of the vaccine by angmenting the magntude of humoral and antigen-specific T cell responses.

NKT cell agonists as adjuvants for Malaria Vaccines

Malaria is an acute febrile illness caused by *Plasmodium* parasites, transmitted to humans by the bite of an infected mosquito vector of the *Anopheles* species. In 2008, the World Health Organization (WHO) estimated that there were about 247 million cases of malaria and nearly 1 million deaths. Most of these incidences occurred among African children-accounting for almost 20% of all childhood deaths in this area. The malaria parasite is characterized by its distinct life cycle involving liver stages immediately after entry into the human host, followed by blood erythrocyte stages. This complex and multistage life cycle provides a large gamut of antigenic variation possibilities, giving the parasite an advantage in terms of immune evasion. However, it is

Table 1 NKT cell agonists used in vaccination strategies

NKT cell activating ligand	Relation to α-GalCer	Disease
α-GalCer, KRN7000		Malaria (Gonzalez-Aseguinolaza et al., 2002), HIV (Huang et al., 2008; Thapa et al., 2009), Influenza (Youn et al., 2007; Ko et al., 2009), Tumors [melanoma (Schmieg et al., 2003)], Hepatitis B virus associated hepatic carcinoma (Shibolet et al., 2003),
α-C-GalCer	C-glycoside analog	Malaria (Schmieg et al., 2010), Influenza (Kopecky-Bromberg et al., 2009)
7DW8-5	Longer acyl chains	Malaria (Li et al., 2010a), HIV (Li et al., 2010a)
KBC-009	Branched acyl chains	Influenza (Lee et al., 2011)

known that Th1 type cytokines such as interferon gamma (IFN-γ) and $CD8^+$ T cells are important in the immune response against the malaria parasite (Schofield et al., 1987). It has also been found that immunization using irradiated sporozoites or viral vectors expressing malaria antigens can provide sterilizing immunity against the liver stages of this parasite (Clyde 1975; Vanderberg et al., 1968). However, there still remains a need for strong and effective adjuvants to elicit a long lasting immune response from these vaccines so as to maximize protection from disease and minimize the requirements for large doses and/or booster doses of the vaccine.

Tsuji and colleagues showed that inclusion of α-GalCer as an adjuvant in a vaccine composed of γ-irradiated sporozoites (γ-spz) significantly enhanced protective immunity within two weeks, as compared to the vaccine alone (Gonzalez-Aseguinolaza et al., 2002). They reported that the parasite load in the livers of mice immunized with γ-spz and 2 μg of α-GalCer was 10 times lower than in mice immunized with γ-spz alone. These results were also corroborated by immunizing mice with other vaccines such as a recombinant Sindbis virus expressing the circumsporozoite (CS) protein or an adenovirus expressing *P. yoelii* CS protein. After ruling out an enhancement of humoral immune responses, the protective effect of α-GalCer was mainly attributed to IFN-γ producing CS-specific $CD8^+$ T cells, while the numbers of interleukin-4 (IL-4) producing T cells was found to be unchanged. Furthermore, the role of NKT cells was confirmed by using $CD1d^{-/-}$ mice which completely lack NKT cells. It was concluded that the adjuvant effect of α-GalCer in combination with malaria vaccines was dependent on NKT cell activation, which was able to boost IFN-γ production by NK cells and memory $CD8^+$ T cells.

An α-C-glycoside analog of α-GalCer was developed by Tsuji's group in 2003 (Schmieg et al., 2003). This analog was shown to have a superior ability to activate NKT cells as compared to α-GalCer, which is known to induce high levels of both Th1 as well as Th2 cytokines *in vivo*. It was also reported that this compound, known as α-C-Galactosylceramide has a strong Th1 bias— making it a more immunogenic adjuvant. This analog was subsequently used in malaria vaccines. This group was able to elucidate the mechanism for immunity against malaria by the intraperitoneal administration of α-GalCer and its C-glycoside analog, followed by challenge with malaria sporozoites (Schmieg et al., 2010). This model proposes that α-C-GalCer is presented by antigen presenting cells (APCs) to NKT cells which are activated and release large amounts of interleukin 12 (IL-12). Notably, this IL-12 production is much higher than that induced by α-GalCer. The NKT cells also produce higher amounts of IFN-γ to supplement the other major source of IFN-γ, namely NK cells. These activated NK cells also produce higher amounts of IFN-γ as a result of increased stimulation by larger amounts of IL-12 released by the NKT cells. These discrete events lead to the overall effect of an immune response that is highly biased toward the Th1 type. This study also reported that α-C-GalCer was able to reduce lung metastasis of melanoma induced in mice by the injection of B16 melanoma cells. This effect was also attributed to the Th1 bias of this c-glycoside derivative of α-GalCer (Schmieg et al., 2003).

In 2010, a study by Li et al., focused on a library of 25 analogs of α-GalCer as potential vaccine adjuvants. The rationale behind developing such an extensive panel was to identify candidates which could induce a specific cytokine profile, either Th1 or Th2, not both as described for α-GalCer, and with greater functionality than the previously reported compound α-C-GalCer. Indeed, a glycolipid compound 7DW8-5 was found to enhance the effectiveness of vaccines against malaria and HIV. Some correlations between the atructure of analogs and the responses elicited by them have also been made: for example, the presence of two benzene rings in the compound was found to dampen the immune response and the presence of longer fatty acyl chains exhibited reduced IFN-γ responses (Li et al., 2010a).

Vaccines against viral pathogens

As per the WHO annual Acquired Immunodeficiency Syndrome (AIDS) epidemic report of 2009, a total of 33.3 million people are currently living with AIDS, a manifestation of HIV infection. HIV/AIDS has been the cause of 1.8 million deaths in the year 2008 alone. With such a large proportion of the world population at risk, the race for developing an effective vaccine against this infection has gained momentum over the past few years. The development of a vaccine against HIV poses several fundamental problems such as an extremely high rate of antigenic variability and suppression of $CD4^+$ T cells infected by the virus. Besides the depletion of conventional T cells, several groups have reported that HIV specifically and selectively targets $CD4^+$ *i*NKT cells ($V\alpha24^+V\beta11^+$) leading to depletion rates that are higher than those observed in conventional $CD4^+$ T cells (Motsinger et al., 2002; Sandberg et al., 2002; van der Vliet et al., 2002; Fleuridor et al., 2003). This has led to an increased focus on vaccines that contain adjuvants to activate NKT cells in the urgent search for effective vaccines.

Needless to say, the activation of NKT cells by α-GalCer as an adjuvant has immense potential in improving current HIV vaccine candidates, since NKT cells are known to have direct cytotoxic anti-viral activity. Research efforts have been directed toward using α-GalCer as an adjuvant for DNA as well as nano-particle based vaccines against HIV. DNA vaccines have been shown to be effective in studies conducted with mice, but the results have proved to be difficult to replicate in non-human primates. This is possibly a result of poor transfection efficiency and/or poor immunogenicity, and has spawned a large search for effective adjuvants for DNA vaccines. Among the many candidate adjuvants that have been tested, α-GalCer is one of the compounds that show promise in this area.

It was found that the co-administration of α-GalCer with a DNA vaccine (pADVAX) encoding the *env* and *gag* proteins of HIV-1 boosted the $CD4^+$ and $CD8^+$ epitope specific IFN-γ responses in mice. It was also demonstrated to induce Gag-specific antibody responses. Additionally, α-GalCer was also shown to have a dose-sparing effect on the DNA vaccine against HIV-1, implying that α-GalCer could bring many more vaccines to the clinic due to its ability to reduce the required immunogenic dose (Huang et al., 2008). Using a prime-boost regime, it was shown that α-GalCer enhanced humoral responses after administration of the DNA vaccine-pADVAX-e/g, a dual promoter vaccine plasmid encoding *env* (gp160) and *gag* of HIV-1. It was reported that antibody responses against HIV-1 Gag protein was much higher than with the DNA vaccine alone. The overall immunoglobulin G (IgG) responses were enhanced with no differences in the subtypes of IgG. Overall, it was concluded that co-administration of the DNA vaccine along with α-GalCer leads to strong humoral responses and a balanced Th2/Th1 response to Gag protein. These findings are in line with previous research which has found that CD1d-mediated activation of NKT cells leads to rapid production of various cytokines and affects the early cytokine milieu. These potent and rapid producers of cytokines can enhance any immune response and boost immunogenicity for a broad range of vaccines against a variety of pathogens.

While there is extensive research being conducted in the field of DNA vaccines, there is also work being directed toward polylactic acid based nano-particle vaccines. Notable among these, is the work published by Thapa et al. (2009). This group reported that the use of nanoparticles along with α-GalCer enhanced antigen presentation by dendritic cells (DCs) and led to strong NKT cell activation. This is in contrast to the use of soluble antigen/α-GalCer vaccines which rapidly cause anergy in NKT cells due to antigen presentation to NKT cells by B cells (Sullivan and Kronenberg 2005; Thapa et al., 2009). The method of vaccination with concomitant activation of NKT cells could be applied to vaccines against various viral antigens such as HIV and influenza virus. Thus vaccines against different pathogens can be improved and made more immunogenic by incorporating α-GalCer as an adjuvant.

Similar ongoing research has focused on vaccines against influenza, which is a major world-wide pathogen. Influenza is a major health concern due to the large number of hospitalizations and deaths that influenza infections cause every year, making the influenza virus an important vaccine target. Moreover, there is a high risk of the emergence of newly reassorted viruses that can rapidly spread among humans. Influenza viruses are now considered to be an even greater health risk due to the possibility of interspecies transmissions (Webster et al., 1995). The recent swine and avian influenza epidemics have created a widespread awareness of the impact of influenza viruses on world population (Nam et al., 2011; Rui-Hua et al., 2011). Consequently, the development of highly immunogenic, and potentially cross-reactive influenza vaccines are critically important in this rapidly evolving field.

A major rationale for utilizing NKT cell activation to boost the effectiveness of an influenza vaccine was shown by Ko et al. (2005). This group was able to underscore the importance of NKT cell responses in vaccines using an ovalbumin (OVA) model. They found that the addition of α-GalCer while immunizing mice against OVA improved the immune response upon subsequent challenge with the OVA peptide. Similar to the effect of α-GalCer in HIV vaccines, they found that α-GalCer as an adjuvant increased the therapeutic efficacy of the vaccine at a lower dose. Moreover, the humoral, cytokine and T cell responses were enhanced and more effective. This group also went on to study the use of α-GalCer as an adjuvant in an influenza virus vaccine, A/PR/8/34 (PR8). They found that when α-GalCer was included in the PR8 vaccine which was nasally administered, the IgG titers were significantly higher than with the vaccine alone. Importantly, the immunoglobulin A (IgA) levels in the nasal washes, lung washes as well as serum from the mice were significantly higher than just the vaccine. In all cases, the response was found to be highly specific to the influenza virus hemagglutinin (HA) antigen. This was the first attempt at using α-GalCer to adjuvant a vaccine for influenza.

A more elaborate study in this field came by Youn et al., in 2007. This study tested the efficacy of a nasal vaccine composed of formalin inactivated PR8 influenza virus along with α-GalCer as an adjuvant in BALB/c mice (Youn et al., 2007). It was found that the use of α-GalCer as an adjuvant lead to reduced viral titers even after immunization with lower doses of the vaccine, while a 10-fold higher dose of the vaccine was required to achieve the same reduction when α-GalCer was not included in the formulation. Once this overall effect was observed, it was found that specific humoral immune responses are significantly enhanced following administration of vaccine with α-GalCer as an adjuvant. The serum as well as lung washes from immunized and challenged mice showed higher titers of PR8-specific IgG as well as IgA. The influenza virus is a pathogen that is most often mucosally contracted, has maximum interaction with the Mucosal-Associated Lymphoid Tissue (MALT) and is recently being targeted with mucosally administered vaccines. This gives special relevance and significance to the enhancement of IgA titers following administration of the PR8 vaccine with α-GalCer. Like antibody responses, immune cell-mediated responses were also found to be stronger with the use of α-GalCer. Mononuclear cells from the spleen and the lymph nodes were found to have a greater proliferative response after administration of the vaccine-adjuvant combination. Interestingly, upon stimulation of these cells with antigen, the Th2 cytokine responses were found to be much higher than with the vaccine alone. IL-4 and IL-5 levels were significantly higher, in contrast to IFN-γ, which was much lower than with the vaccine alone. The

lower viral titers and higher survival rates corroborate the fact that Th2 responses are important in immune responses to influenza. This Th2 shift of the cytokine environment favors a strong mucosal IgA response, which is critical for a pathogen like the influenza virus (Kamijuku et al., 2008). This study also reported that long-term memory T cell responses were more robust in mice immunized with adjuvant containing vaccine. In 2009, the analog α-C-GalCer was also reported to have similar effects when incorporated into a vaccine for influenza consisting of PR8 with a truncated NS1 region (Kopecky-Bromberg et al., 2009). α-C-GalCer is known to have a stronger bias toward Th1 responses as compared to α-GalCer. Indeed it was reported that IFN-γ dependent $CD8^+$ T cell responses played an important role in enhancing immune response with the use of this analog as an adjuvant. This may seem counterintuitive in lieu of the previously discussed study by Youn et al. (Youn et al., 2007); however, $CD8^+$ specific responses were not studied in the former, and α-GalCer was not compared to α-C-GalCer in the latter study. This renders a direct evaluation of the mechanism of the NKT cell activating adjuvant unfeasible. Only further study of $CD4^+$ and $CD8^+$ responses with the use of α-GalCer and its analogs, including direct side-by-side comparisons, can help tease out the precise role of cytotoxicity, cytokines and Th1/Th2 responses in the effect of these adjuvants.

Kamijuku et al. (2008) used fluorescently labeled α-GalCer to study its localization and found that following administration of mucosal vaccines, the adjuvant α-GalCer was highly taken up by DCs in the nasal-associated lymphoid tissue (NALT) and poorly so by B cells. This was postulated as a mechanism by which the α-GalCer leads to NKT cell activation and not anergy, as seen by systemic administration of α-GalCer. It was also reported that this accumulation of α-GalCer bearing DCs in the NALT led to further recruitment of NKT cells in a CXCL16/CXCR6— chemokine/chemokine receptor dependent fashion. Thus α-GalCer is not only able to activate NKT cells, but through its effects on DCs, is able to alter the spatiotemporal distribution of NKT cells in the mucosa.

Besides α-C-GalCer, other analogs of α-GalCer have also been evaluated for their potential as adjuvants. Notably, NKT cell activation by two compounds, KBC-007 and KBC-009, which have different branched-chain lengths, was recently characterized (Lee et al., 2011). KBC-009 was shown to induce both Th1 and Th2 cytokines, while responses to KBC-007 exhibited a Th2 bias. It was found that KBC-009 was more efficient at enhancing immune responses after immunization with the PR8 vaccine, compared to KBC-007. These findings warrant development of α-GalCer analogs with branched acyl chains since these may be better for use as adjuvants. This report also highlights the complexity of the role of Th1/Th2 responses in the adjuvant effect of NKT cell agonists. Taken together, these studies might be an indication that Th2 responses are required for IgA secretion, but the Th1 cytokine IFN-γ is required for priming $CD8^+$ T cell cytotoxic responses. Both of these responses may be essential for the effective clearance of infection, potentially explaining the observation that an analog which induces both Th1 and Th2 responses is more effective than one that induces Th2 type cytokines alone. This also strongly suggests that both humoral and cell-mediated immune responses are equally important and necessary for enhancing effectiveness of mucosal vaccines through adjuvantation using NKT cell agonists.

Thus NKT cell activating ligands have shown promise for conventional peptide-based vaccines, mucosally delivered vaccines, DNA vaccines as well as nano-particle based vaccines. α-GalCer and its analogs could soon facilitate many vaccines against a broad array of parasitic and viral pathogens due to their enhancement of immune responses through their ability to modulate cytokine levels, humoral responses as well as CTL responses.

Adjuvants for vaccines against tumors

The original discovery of the potent anti-tumor function of NKT cells stemmed from the observation that α-GalCer injected into mice led to a striking reduction in B16 melanoma tumor burden (Kobayashi et al., 1995; Nakagawa et al., 2000). Later, it was also found that cancer patients have reduced numbers of NKT cells, independent of tumor type and load (Molling et al., 2005). Since the identification of NKT cells, there have been innumerable efforts to utilize them as a therapeutic strategy against tumors. The systemic introduction of α-GalCer can lead to anergy of NKT cells, due to overstimulation (Sullivan and Kronenberg, 2005; Uldrich et al., 2005). On the other hand, vaccines directed toward tumor antigens have been impeded by the requirement of very high doses of antigen, weak immune responses and consequent residual tumor burdens leading to either incomplete remission of disease, or rapid relapse (Bauer et al., 2011). This has fostered research in the use of NKT cell agonists as adjuvants in vaccines targeted against tumor antigens.

The role of NKT cells in immune responses to cancer has been widely studied. It has been shown that NKT cell mediated tumor cytolytic responses, at least in mice, are highly organ specific (Crowe et al., 2005). This is of particular importance in the context of anti-tumor responses because $CD4^-$ NKT cells from the liver are known to reject tumors, in contrast to other subsets of NKT cells such as those from the thymus and spleen (Crowe et al., 2005). The functional roles of $CD4^-CD8^-$, $CD4^+$ and $CD8^+$ NKT cells have been difficult to study, due to differences in human and mouse NKT cell subsets (Lee et al., 2002). Mice lack the $CD8^+$ NKT cell subset. Almost all studies involving the use of NKT cell agonists as vaccine adjuvants have been conducted using mouse models. Consequently, little is known about the potential anti-tnmor role of different NKT cell subsets if α-GalCer and its analogs are used as vaccine adjuvants in

humans. There have also been recent reports on $CD4^+$ regulatory NKT cells, although their contribution to the adjuvant effect of NKT cell agonists remains to be elucidated (Osada et al., 2005).

Among some of the earliest studies, it was found that a reduction in melanoma metastases following B16 melanoma cell injections could be achieved using systemic administration of α-C-GalCer to mice (Schmieg et al., 2003). Among other advances studies in the field, is the use of α-GalCer as an adjuvant for a DC based vaccine against hepatocellular carcinoma (Shibolet et al., 2003). More specifically, the vaccine was used to target Hepatitis B Virus (HBV) associated hepatocellular carcinoma. These tumor cells express the HBV surface antigen (HBsAg). This antigen was loaded on to DCs, along with α-GalCer as an adjuvant and was found to cause rapid remission from disease in mice. The anti-tumor effect was attributed mainly to cytotoxic $CD8^+$ T cell responses. However, IFN-γ and IL-12 are also involved in the anti-tumor response. The adjuvanted vaccine was found to be significantly better than the vaccine alone in terms of mean survival, as well as tumor load. Another strategy that has been employed to target lymphomas is the use of autologous tumor specific antigens (Chung et al., 2007). These antigens are highly patient specific and effective, but their poor immunogenicity is a major obstacle to their application. This was overcome by the use of α-GalCer loaded A20 lymphoma cells in a mouse model. The vaccine was found to elicit effective immune responses to lymphoma in mice in a $CD4^+$ T cell dependent manner. Together these studies also shed light on the fact that different immune responses ($CD4^+$ and $CD8^+$ T cells) are elicited following administration of vaccines with α-GalCer and the specific response elicited may depend upon the type of vaccine, as well as the tumor type.

Besides the use of autologous tumor cells and DCs as APCs, attempts have also been directed toward identifying other APCs (Petersen et al., 2010). For example, it has been shown that primary B cells transduced with adenovirus encoding truncated Her-2/neu tumor-specific antigen along with α-GalCer was effective at raising specific antibody responses (Kim et al., 2008). $CD4^+$ T cells, $CD8^+$ T cells and NK cells were found to be important for the anti-tumor response elicited by the vaccine. The problem of NKT cell anergy due to antigen presentation by B cells did not pose a problem in this model and could, in fact, be further evidence of context-dependent activation of NKT cells and subsequent fine tuning of innate and adaptive immune responses. Another study also used the Her-2/neu expressing adenovirus transduction strategy, but the APCs used in this case were Myeloid Derived Suppressor Cells (MDSC) (Ko et al., 2009). When these cells expressing tumor antigen were loaded with α-GalCer, they did not suppress $CD8^+$ T cell responses or induce Forkhead Box P3 (FoxP3) expressing regulatory T (Treg) cells. In fact, the vaccine was found to enhance tumor-specific CTL responses, and the MDSCs underwent phenotypic changes and acted as potent immunogenic APCs (Ko et al., 2009). In 2010, Kim et al. used a combination strategy for an anti-tumor vaccine (Kim et al., 2010). The regimen consisted of a priming dose of DNA vaccine- DCs transduced to express Human Papilloma Virus Type 16- E7 antigen along with α-GalCer as an adjuvant. However, the subsequent boosters consisted of DCs loaded with only E7 antigen. They reported effective anti-tumor responses with the use of a DNA vaccine boosted with α-GalCer followed by an antigen-loaded DC-based booster vaccine. Besides these strategies, there have also been α-GalCer and its analogs have also been included in monoclonal antibody therapy against tumors for effective anti-tumor immunotherapy (Teng et al., 2007).

Most of these studies have been conducted in mouse models. Much work has been done in testing the effects of direct administration of NKT cells activating ligands on non-human primates as well as human patients (Giaccone et al., 2002; Matangkasombut et al., 2008). However, the specific strategy of using these compounds as vaccine adjuvants remains to be tested on higher animals and human subjects. Several studies are currently underway and will pave the way for translational application of these strategies in the near future.

Concluding remarks

To date, the use of α-GalCer and its analogs as adjuvants in vaccines has proved to be a huge success in many different disease settings. NKT cell agonists have been shown to improve the efficacy of vaccines against malaria, HIV as well as influenza. This strategy may also be applicable to other pathogens such as Hepatitis C Virus (HCV) and warrants further investigation. The use of these adjuvants has also been extended to anti-tumor vaccines and has shown promise in many different types of tumors ranging from breast cancer to lymphomas. Different mechanisms of action have been reported for these NKT-specific adjuvants, including humoral immunity, Th1/Th2 responses, $CD4^+$ T cell responses, $CD8^+$ T cell mediated cytotoxicity and even NK cell activity (Schmieg et al., 2003; Ko et al., 2005; Huang et al., 2008; Kopecky-Bromberg et al., 2009; Schmieg et al., 2010; Lee et al., 2011). The precise mechanism required for effective responses depends upon the design of the vaccine and the disease model under study. Another exciting aspect of the use of α-GalCer and its analogs as vaccine adjuvants is that they have ushered in new generation vaccines such as DNA vaccines, DC-based vaccines and nano-particle based vaccines. The activation of NKT cells has helped overcome the limitations imposed by high dose requirement and poor immunogenicity, bringing them closer to reality than ever before.

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Abbreviations

Natural killer T (NKT); α-galactosylceramide (α-GalCer); T cell receptor (TCR); γ-irradiated sporozoites (γ-spz); dendritic cells (DCs); isoglobotrihexosylceramide (iGb3); glycosylphosphatidylinositol (GPI); World Health Organization (WHO); major histocompatibility Complex (MHC); interferon gamma (IFN-γ); antigen presenting cells (APCs); T Helper (Th); human immunodeficiency Virus (HIV); circumsporozoite (CS); interleukin-4 (IL-4); immunoglobulin G (IgG); immunoglobulin A (IgA); ovalbumin (OVA); hemagglutinin (HA); mucosal associated lymphoid tissue (MALT); nasal associated lymphoid tissue (NALT); hepatitis B surface antigen (HBsAg); myeloid derived suppressor cells (MDSC); T-regulatory cell (Treg); Forkhead Box P3 (FoxP3)

References

Bauer C, Dauer M, Saraj S, Schnurr M, Bauernfeind F, Sterzik A, Junkmann J, Jakl V, Kiefl R, Oduncu F, Emmerich B, Mayr D, Mussack T, Bruns C, Rüttinger D, Conrad C, Jauch K W, Endres S, Eigler A (2011). Dendritic cell-based vaccination of patients with advanced pancreatic carcinoma: results of a pilot study. Cancer Immunol Immunother, 60(8): 1097–1107

Blauvelt M L, Khalili M, Jaung W, Paulsen J, Anderson A C, Brian Wilson S, Howell A R (2008). Alpha-S-GalCer: synthesis and evaluation for iNKT cell stimulation. Bioorg Med Chem Lett, 18(24): 6374–6376

Burdin N, Brossay L, Koezuka Y, Smiley S T, Grusby M J, Gui M, Taniguchi M, Hayakawa K, Kronenberg M (1998). Selective ability of mouse CD1 to present glycolipids: alpha-galactosylceramide specifically stimulates V alpha 14^+ NK T lymphocytes. J Immunol, 161(7): 3271–3281

Carnaud C, Lee D, Donnars O, Park S H, Beavis A, Koezuka Y, Bendelac A (1999). Cutting edge: Cross-talk between cells of the innate immune system: NKT cells rapidly activate NK cells. J Immunol, 163(9): 4647–4650

Chung Y, Qin H, Kang C Y, Kim S, Kwak L W, Dong C (2007). An NKT-mediated autologous vaccine generates CD4 T-cell dependent potent antilymphoma immunity. Blood, 110(6): 2013–2019

Clyde D F (1975). Immunization of man against falciparum and vivax malaria by use of attenuated sporozoites. Am J Trop Med Hyg, 24(3): 397–401

Courtney A N, Thapa P, Singh S, Wishahy A M, Zhou D, Sastry K J (2011). Intranasal but not intravenous delivery of the adjuvant alpha-galactosylceramide permits repeated stimulation of natural killer T cells in the lung. Eur J Immunol, 41(11):3312–3322

Crowe N Y, Coquet J M, Berzins S P, Kyparissoudis K, Keating R, Pellicci D G, Hayakawa Y, Godfrey D I, Smyth M J (2005). Differential antitumor immunity mediated by NKT cell subsets *in vivo*. J Exp Med, 202(9): 1279–1288

Fleuridor R, Wilson B, Hou R, Landay A, Kessler H, Al-Harthi L (2003). CD1d-restricted natural killer T cells are potent targets for human immunodeficiency virus infection. Immunology, 108(1): 3–9

Fowlkes B J, Kruisbeek A M, Ton-That H, Weston M A, Coligan J E, Schwartz R H, Pardoll D M (1987). A novel population of T-cell receptor alpha beta-bearing thymocytes which predominantly expresses a single V beta gene family. Nature, 329(6136): 251–254

Giaccone G, Punt C J, Ando Y, Ruijter R, Nishi N, Peters M, von Blomberg B M, Scheper R J, van der Vliet H J, van den Eertwegh A J, Roelvink M, Beijnen J, Zwierzina H, Pinedo H M (2002). A phase I study of the natural killer T-cell ligand alpha-galactosylceramide (KRN7000) in patients with solid tumors. Clin Cancer Res, 8(12): 3702–3709

Gonzalez-Aseguinolaza G, Van Kaer L, Bergmann C C, Wilson J M, Schmieg J, Kronenberg M, Nakayama T, Taniguchi M, Koezuka Y, Tsuji M (2002). Natural killer T cell ligand alpha-galactosylceramide enhances protective immunity induced by malaria vaccines. J Exp Med, 195(5): 617–624

Hogan A E, O'Reilly V, Dunne M R, Dere R T, Zeng S G, O'Brien C, Amu S, Fallon P G, Exley M A, O'Farrelly C, Zhu X, Doherty D G (2011). Activation of human invariant natural killer T cells with a thioglycoside analogue of α-galactosylceramide. Clin Immunol, 140 (2): 196–207

Huang Y, Chen A, Li X, Chen Z, Zhang W, Song Y, Gurner D, Gardiner D, Basu S, Ho D D, Tsuji M (2008). Enhancement of HIV DNA vaccine immunogenicity by the NKT cell ligand, alpha-galactosylceramide. Vaccine, 26(15): 1807–1816

Joyce S, Woods A S, Yewdell J W, Bennink J R, De Silva A D, Boesteanu A, Balk S P, Cotter R J, Brutkiewicz R R (1998). Natural ligand of mouse CD1d1: cellular glycosylphosphatidylinositol. Science, 279(5356): 1541–1544

Kamijuku H, Nagata Y, Jiang X, Ichinohe T, Tashiro T, Mori K, Taniguchi M, Hase K, Ohno H, Shimaoka T, Yonehara S, Odagiri T, Tashiro M, Sata T, Hasegawa H, Seino K I (2008). Mechanism of NKT cell activation by intranasal coadministration of alpha-galactosylceramide, which can induce cross-protection against influenza viruses. Mucosal Immunol, 1(3): 208–218

Kawakami K, Kinjo Y, Yara S, Koguchi Y, Uezu K, Nakayama T, Taniguchi M, Saito A (2001). Activation of Valpha14(+) natural killer T cells by alpha-galactosylceramide results in development of Th1 response and local host resistance in mice infected with Cryptococcus neoformans. Infect Immun, 69(1): 213–220

Kawano T, Cui J, Koezuka Y, Toura I, Kaneko Y, Motoki K, Ueno H, Nakagawa R, Sato H, Kondo E, Koseki H, Taniguchi M (1997). CD1d-restricted and TCR-mediated activation of valpha14 NKT cells by glycosylceramides. Science, 278(5343): 1626–1629

Kim D, Hung C F, Wu T C, Park Y M (2010). DNA vaccine with α-galactosylceramide at prime phase enhances anti-tumor immunity after boosting with antigen-expressing dendritic cells. Vaccine, 28 (45): 7297–7305

Kim Y J, Ko H J, Kim Y S, Kim D H, Kang S, Kim J M, Chung Y, Kang C Y (2008). Alpha-Galactosylceramide-loaded, antigen-expressing B cells prime a wide spectrum of antitumor immunity. Int J Cancer, 122 (12): 2774–2783

Kinjo Y, Tupin E, Wu D, Fujio M, Garcia-Navarro R, Benhnia M R, Zajonc D M, Ben-Menachem G, Ainge G D, Painter G F, Khurana A, Hoebe K, Behar S M, Beutler B, Wilson I A, Tsuji M, Sellati T J, Wong C H, Kronenberg M (2006). Natural killer T cells recognize

diacylglycerol antigens from pathogenic bacteria. Nat Immunol, 7(9): 978–986

Kinjo Y, Wu D, Kim G, Xing G W, Poles M A, Ho D D, Tsuji M, Kawahara K, Wong C H, Kronenberg M (2005). Recognition of bacterial glycosphingolipids by natural killer T cells. Nature, 434 (7032): 520–525

Ko H J, Lee J M, Kim Y J, Kim Y S, Lee K A, Kang C Y (2009). Immunosuppressive myeloid-derived suppressor cells can be converted into immunogenic APCs with the help of activated NKT cells: an alternative cell-based antitumor vaccine. J Immunol, 182(4): 1818–1828

Ko S Y, Ko H J, Chang W S, Park S H, Kweon M N, Kang C Y (2005). alpha-Galactosylceramide can act as a nasal vaccine adjuvant inducing protective immune responses against viral infection and tumor. J Immunol, 175(5): 3309–3317

Kobayashi E, Motoki K, Uchida T, Fukushima H, Koezuka Y (1995). KRN7000, a novel immunomodulator, and its antitumor activities. Oncol Res, 7(10–11): 529–534

Kopecky-Bromberg S A, Fraser K A, Pica N, Carnero E, Moran T M, Franck R W, Tsuji M, Palese P (2009). Alpha-C-galactosylceramide as an adjuvant for a live attenuated influenza virus vaccine. Vaccine, 27(28): 3766–3774

Koseki H, Asano H, Inaba T, Miyashita N, Moriwaki K, Lindahl K F, Mizutani Y, Imai K, Taniguchi M (1991). Dominant expression of a distinctive $V14^{+}$ T-cell antigen receptor alpha chain in mice. Proc Natl Acad Sci USA, 88(17): 7518–7522

Lee P T, Benlagha K, Teyton L, Bendelac A (2002). Distinct functional lineages of human V(alpha)24 natural killer T cells. J Exp Med, 195 (5): 637–641

Lee Y S, Lee K A, Lee J Y, Kang M H, Song Y C, Baek D J, Kim S, Kang C Y (2011). An α-GalCer analogue with branched acyl chain enhances protective immune responses in a nasal influenza vaccine. Vaccine, 29(3): 417–425

Li X, Fujio M, Imamura M, Wu D, Vasan S, Wong C H, Ho D D, Tsuji M (2010a). Design of a potent CD1d-binding NKT cell ligand as a vaccine adjuvant. Proc Natl Acad Sci USA, 107(29): 13010–13015

Li Y, Girardi E, Wang J, Yu E D, Painter G F, Kronenberg M, Zajonc D M (2010b). The Vα14 invariant natural killer T cell TCR forces microbial glycolipids and CD1d into a conserved binding mode. J Exp Med, 207(11): 2383–2393

Lu X, Song L, Metelitsa L S, Bittman R (2006). Synthesis and evaluation of an alpha-C-galactosylceramide analogue that induces Th1-biased responses in human natural killer T cells. ChemBioChem, 7(11): 1750–1756

Makino Y, Kanno R, Ito T, Higashino K, Taniguchi M (1995). Predominant expression of invariant V alpha 14 + TCR alpha chain in $NK1.1^{+}$ T cell populations. Int Immunol, 7(7): 1157–1161

Matangkasombut P, Pichavant M, Yasumi T, Hendricks C, Savage P B, Dekruyff R H, Umetsu D T (2008). Direct activation of natural killer T cells induces airway hyperreactivity in nonhuman primates. J Allergy Clin Immunol, 121(5): 1287–1289

Mattner J, Debord K L, Ismail N, Goff R D, Cantu C 3rd, Zhou D, Saint-Mezard P, Wang V, Gao Y, Yin N, Hoebe K, Schneewind O, Walker D, Beutler B, Teyton L, Savage P B, Bendelac A (2005). Exogenous and endogenous glycolipid antigens activate NKT cells during microbial infections. Nature, 434(7032): 525–529

Miyamoto K, Miyake S, Yamamura T (2001). A synthetic glycolipid prevents autoimmune encephalomyelitis by inducing TH2 bias of natural killer T cells. Nature, 413(6855): 531–534

Molling J W, Kölgen W, van der Vliet H J, Boomsma M F, Kruizenga H, Smorenburg C H, Molenkamp B G, Langendijk J A, Leemans C R, von Blomberg B M, Scheper R J, van den Eertwegh A J (2005). Peripheral blood IFN-gamma-secreting $Valpha24^{+}$ $Vbeta11^{+}$ NKT cell numbers are decreased in cancer patients independent of tumor type or tumor load. Int J Cancer, 116(1): 87–93

Motsinger A, Haas D W, Stanic A K, Van Kaer L, Joyce S, Unutmaz D (2002). CD1d-restricted human natural killer T cells are highly susceptible to human immunodeficiency virus 1 infection. J Exp Med, 195(7): 869–879

Nakagawa R, Serizawa I, Motoki K, Sato M, Ueno H, Iijima R, Nakamura H, Shimosaka A, Koezuka Y (2000). Antitumor activity of alpha-galactosylceramide, KRN7000, in mice with the melanoma B16 hepatic metastasis and immunohistological study of tumor infiltrating cells. Oncol Res, 12(2): 51–58

Nam J H, Kim E H, Song D, Choi Y K, Kim J K, Poo H (2011). Emergence of mammalian species-infectious and -pathogenic avian influenza H6N5 virus with no evidence of adaptation. J Virol, 7 (23):3281–3286

Natori T, Akimoto K, Motoki K, Koezuka Y, Higa T (1997). Development of KRN7000, derived from agelasphin produced by okinawan sponge. Nihon Yakurigaku Zasshi, 110 Suppl 163P–68P

Niemeyer M, Darmoise A, Mollenkopf H J, Hahnke K, Hurwitz R, Besra G S, Schaible U E, Kaufmann S H (2008). Natural killer T-cell characterization through gene expression profiling: an account of versatility bridging T helper type 1 (Th1), Th2 and Th17 immune responses. Immunology, 123(1): 45–56

Osada T, Morse M A, Lyerly H K, Clay T M (2005). *Ex vivo* expanded human $CD4^{+}$ regulatory NKT cells suppress expansion of tumor antigen-specific CTLs. Int Immunol, 17(9): 1143–1155

Parekh V V, Wilson M T, Olivares-Villagómez D, Singh A K, Wu L, Wang C R, Joyce S, Van Kaer L (2005). Glycolipid antigen induces long-term natural killer T cell anergy in mice. J Clin Invest, 115(9): 2572–2583

Petersen T R, Sika-Paotonu D, Knight D A, Dickgreber N, Farrand K J, Ronchese F, Hermans I F (2010). Potent anti-tumor responses to immunization with dendritic cells loaded with tumor tissue and an NKT cell ligand. Immunol Cell Biol, 88(5): 596–604

Porcelli S, Gerdes D, Fertig A M, Balk S P (1996). Human T cells expressing an invariant V alpha 24-J alpha Q TCR alpha are CD4- and heterogeneous with respect to TCR beta expression. Hum Immunol, 48(1–2): 63–67

Roberts T J, Sriram V, Spence P M, Gui M, Hayakawa K, Bacik I, Bennink J R, Yewdell J W, Brutkiewicz R R (2002). Recycling CD1d1 molecules present endogenous antigens processed in an endocytic compartment to NKT cells. J Immunol, 168(11): 5409–5414

Rui-Hua Z, Hong-Yu C, Ming-Ju X, Kai L, Hua-Lan C, Cun-Lian W, Dong W, Cun-Xin L, Tong X (2011). Molecular characterization and pathogenicity of swine influenza H9N2 subtype virus A/swine/HeBei/012/2008/(H9N2). Acta Virol, 55(3): 219–226

Sandberg J K, Fast N M, Palacios E H, Fennelly G, Dobroszycki J, Palumbo P, Wiznia A, Grant R M, Bhardwaj N, Rosenberg M G, Nixon D F (2002). Selective loss of innate $CD4(^{+})$ V alpha 24 natural killer T cells in human immunodeficiency virus infection. J Virol, 76

(15): 7528–7534

Schmieg J, Yang G, Franck R W, Tsuji M (2003). Superior protection against malaria and melanoma metastases by a C-glycoside analogue of the natural killer T cell ligand alpha-Galactosylceramide. J Exp Med, 198(11): 1631–1641

Schmieg J, Yang G, Franck R W, Tsuji M (2010). A multifactorial mechanism in the superior antimalarial activity of alpha-C-GalCer. J Biomed Biotechnol, 2010: 283612

Schofield L, Villaquiran J, Ferreira A, Schellekens H, Nussenzweig R, Nussenzweig V (1987). Gamma interferon, $CD8^+$ T cells and antibodies required for immunity to malaria sporozoites. Nature, 330 (6149): 664–666

Shibolet O, Alper R, Zlotogarov L, Thalenfeld B, Engelhardt D, Rabbani E, Ilan Y (2003). NKT and CD8 lymphocytes mediate suppression of hepatocellular carcinoma growth via tumor antigen-pulsed dendritic cells. Int J Cancer, 106(2): 236–243

Silk J D, Hermans I F, Gileadi U, Chong T W, Shepherd D, Salio M, Mathew B, Schmidt R R, Lunt S J, Williams K J, Stratford I J, Harris A L, Cerundolo V (2004). Utilizing the adjuvant properties of CD1d-dependent NK T cells in T cell-mediated immunotherapy. J Clin Invest, 114(12): 1800–1811

Sriram V, Du W, Gervay-Hague J, Brutkiewicz R R (2005). Cell wall glycosphingolipids of Sphingomonas paucimobilis are CD1d-specific ligands for NKT cells. Eur J Immunol, 35(6): 1692–1701

Sullivan B A, Kronenberg M (2005). Activation or anergy: NKT cells are stunned by alpha-galactosylceramide. J Clin Invest, 115(9): 2328–2329

Teng M W, Westwood J A, Darcy P K, Sharkey J, Tsuji M, Franck R W, Porcelli S A, Besra G S, Takeda K, Yagita H, Kershaw M H, Smyth M J (2007). Combined natural killer T-cell based immunotherapy eradicates established tumors in mice. Cancer Res, 67(15): 7495–7504

Thapa P, Zhang G, Xia C, Gelbard A, Overwijk W W, Liu C, Hwu P, Chang D Z, Courtney A, Sastry J K, Wang P G, Li C, Zhou D (2009). Nanoparticle formulated alpha-galactosylceramide activates NKT cells without inducing anergy. Vaccine, 27(25–26): 3484–3488

Uldrich A P, Crowe N Y, Kyparissoudis K, Pellicci D G, Zhan Y, Lew A M, Bouillet P, Strasser A, Smyth M J, Godfrey D I (2005). NKT cell stimulation with glycolipid antigen *in vivo*: costimulation-dependent expansion, Bim-dependent contraction, and hyporesponsiveness to further antigenic challenge. J Immunol, 175(5): 3092–3101

van der Vliet H J, von Blomberg B M, Hazenberg M D, Nishi N, Otto S A, van Benthem B H, Prins M, Claessen F A, van den Eertwegh A J, Giaccone G, Miedema F, Scheper R J, Pinedo H M (2002). Selective decrease in circulating V alpha 24^+V beta 11^+ NKT cells during HIV type 1 infection. J Immunol, 168(3): 1490–1495

Vanderberg J P, Nussenzweig R S, Most H, Orton C G (1968). Protective immunity produced by the injection of x-irradiated sporozoites of Plasmodium berghei. II. Effects of radiation on sporozoites. J Parasitol, 54(6): 1175–1180

Velmourougane G, Raju R, Bricard G, Im J S, Besra G S, Porcelli S A, Howell A R (2009). Synthesis and evaluation of an acyl-chain unsaturated analog of the Th2 biasing, immunostimulatory glycolipid, OCH. Bioorg Med Chem Lett, 19(13): 3386–3388

Webster R G, Sharp G B, Claas E C (1995). Interspecies transmission of influenza viruses. Am J Respir Crit Care Med, 152(4 Pt 2): S25–S30

Yamaguchi Y, Motoki K, Ueno H, Maeda K, Kobayashi E, Inoue H, Fukushima H, Koezuka Y (1996). Enhancing effects of (2S,3S,4R)-1-O-(alpha-D-galactopyranosyl)-2-(N-hexacosanoylamino) -1,3,4-octadecanetriol (KRN7000) on antigen-presenting function of antigen-presenting cells and antimetastatic activity of KRN7000-pretreated antigen-presenting cells. Oncol Res, 8(10–11): 399–407

Yang G, Schmieg J, Tsuji M, Franck R W (2004). The C-glycoside analogue of the immunostimulant alpha-galactosylceramide (KRN7000): synthesis and striking enhancement of activity. Angew Chem Int Ed Engl, 43(29): 3818–3822

Yoshiga Y, Goto D, Segawa S, Ohnishi Y, Matsumoto I, Ito S, Tsutsumi A, Taniguchi M, Sumida T (2008). Invariant NKT cells produce IL-17 through IL-23-dependent and -independent pathways with potential modulation of Th17 response in collagen-induced arthritis. Int J Mol Med, 22(3): 369–374

Yoshimoto T, Bendelac A, Watson C, Hu-Li J, Paul W E (1995). Role of $NK1.1^+$ T cells in a TH2 response and in immunoglobulin E production. Science, 270(5243): 1845–1847

Youn H J, Ko S Y, Lee K A, Ko H J, Lee Y S, Fujihashi K, Boyaka P N, Kim S H, Horimoto T, Kweon M N, Kang C Y (2007). A single intranasal immunization with inactivated influenza virus and alpha-galactosylceramide induces long-term protective immunity without redirecting antigen to the central nervous system. Vaccine, 25(28): 5189–5198

Yu E D, Girardi E, Wang J, Zajonc D M (2011). Cutting Edge: Structural basis for the recognition of β-linked glycolipid antigens by invariant NKT cells. J Immunol, 187(5): 2079–2083

Zhou D, Mattner J, Cantu C 3rd, Schrantz N, Yin N, Gao Y, Sagiv Y, Hudspeth K, Wu Y P, Yamashita T, Teneberg S, Wang D, Proia R L, Levery S B, Savage P B, Teyton L, Bendelac A (2004). Lysosomal glycosphingolipid recognition by NKT cells. Science, 306(5702): 1786–1789