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Clinical development of a novel CD1d-binding NKT cell ligand as a vaccine adjuvant

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Abstract

Natural killer T (NKT) cells are known to play a role against certain microbial infections, including malaria and HIV, two major global infectious diseases. Strategies that harness and amplify the immunotherapeutic potential of NKT cells can serve as powerful tools in the fight against such diseases. 7DW8-5, a novel glycolipid, may be one such tool. The interaction of 7DW8-5 with CD1d molecules induces activation of NKT cells, thereby activating various immune-competent cells including dendritic cells (DCs) to provide a significant adjuvant effect for several vaccines. This review discusses the discovery and characterization of 7DW8-5 and the practical considerations of its preclinical and clinical development as a potential glycolipid adjuvant for candidate malaria and HIV vaccines.

Keywords

NKT cell; Glycolipid; CD1d; 7DW8-5; HIV; Malaria; Vaccine; Adjuvant

1. Introduction

1.1. NKT cells, CD1d molecules and glycolipids

Natural killer T cells (NKT) are distinct from conventional T cells and natural killer (NK) cells [1] as they are characterized by the expression of both a T cell antigen receptor (TCR) and NK1.1 (NKR-P1 or CD161c), a C-lectin type natural killer (NK) receptor. As the TCR is invariant for the majority of murine and human NKT cells, encoded by the V α 14 and J α 18 gene segments [2], or V α 24 and J α 18 segments [1], respectively, these NKT cells are called invariant NKT (*i*NKT) cells. The V β repertoire utilized by NKT cells is somewhat more diverse [2–4]. Murine NKT cells usually belong to either CD4[−]CD8[−] (double negative) or CD4⁺CD8[−] sub-populations [2–4]. Human NKT cells are more heterogeneous and a significant percentage of human NKT cells are CD4[−]CD8⁺, both in the blood and the liver [5].

The only characterized ligand of NKT cells to date is CD1d, an MHC class I-like molecule [2,6–8]. While the CD1d protein is not encoded by the MHC gene locus [6,7], this molecule

associates non-covalently with β 2-microglobulin and exhibits limited, yet significant, homology as well as overall structural similarity with MHC class I heavy chains [6,7]. CD1d is constitutively expressed by many cells, including dendritic cells (DCs), macrophages, and B and T lymphocytes [9,10]. Although all iNKT cells recognize and are restricted by CD1d molecules, some NKT cells having more diversified TCR also recognize CD1d [1]. There is a population of NKT cells that does not depend on the recognition of CD1d molecules and known as non-CD1d-restricted NKT cells [1].

A synthetic glycolipid designated α -galactosylceramide (α -GalCer), originally identified in an Okinawan marine sponge by a research group at the Kirin Brewery Co. in Japan [1,11], was shown to bind to CD1d. This was documented by surface plasmon resonance using immobilized α -GalCer and soluble murine [12,13] or human CD1d [12]. This glycolipid, when presented by the CD1d molecule, activates both murine and human NKT cells *in vivo* and *in vitro* [14–17]. X-ray crystallography studies have revealed that the lipid portion of the glycolipid fits tightly into the CD1d binding groove, wherein the sphingosine chain associates with one pocket in the groove and the longer acyl chain anchors within a separate pocket [18,19]. A more recent structural study using a tri-molecular complex consisting of CD1d-glycolipid and V α 24 TCR confirmed that the galactose ring extends above the surface of the lipid-binding groove, and thereby is exposed for recognition by the TCR of NKT cells [20].

Eliminating or exchanging this moiety with various sugars has been shown to either diminish or abrogate activity [14], indicating the importance of the galactose head group to α -GalCer function. We and others have shown that the α -anomeric conformation of the glycolipid as well as the equatorial configuration of the 2-hydroxyl group of the sugar moiety and the 3-hydroxyl group of the phytosphingosine are also crucial for α -GalCer to bind CD1d molecules and to activate NKT cells through their TCR [1,13,21,22]. One study has shown that, albeit at lower potency than α -anomeric GalCer, β -anomeric GalCer can induce CD1d-dependent biological activities in mice [23]. Earlier research has shown that α -GalCer has been amenable to modification in the acyl tails of the molecule, since varying the hydrocarbon lengths and/or introducing unsaturation in the fatty acid chains [24,25], as well as the truncation of the fatty acid chain from 24 to 2 carbons [21] did not significantly affect mouse NKT cell responses. Interestingly, a compound that has only 9 carbons at the sphingosine chain, OCH, is shown to skew the cytokine release profile towards Th-2 cytokines [26]. In contrast, we have previously found that a C-glycoside analog of α -GalCer, α -C-GalCer, preferentially stimulates a Th1-type response in mice [27,28]. This analog, in comparison to α -GalCer, consistently stimulated prolonged production of IFN- γ , increased production of IL-12, and decreased production of the Th2 cytokine IL-4.

1.2. Function of glycolipid-activated NKT cells and their adjuvant effect

A number of studies have documented the protective role of α -GalCer-activated NKT cells in anti-tumor immunity [29,30], autoimmune diseases [31–34] and infectious diseases [35–39]. α -GalCer and α -C-GalCer have recently been shown to induce full maturation of DCs, as determined by an increased expression of co-stimulatory molecules, which include CD40, CD80 and CD86, as well as MHC class II molecules on DCs [40,41]. The presence

of NKT cells is required in order for the glycolipids to activate DCs. Although the precise mechanisms for this DC maturation process and for the functional consequence of the DC maturation are still unknown, it is thought to involve multi-factorial pathways in order to lead to full maturation/activation of DCs [42].

To date, several clinical trials have been conducted using α -GalCer primarily for the treatment of cancer, but also as a potential therapeutic agent against Hepatitis B and Hepatitis C [43–48]. Direct intravenous (IV) administration of α -GalCer did not result in a noticeable clinical benefit against cancer, hepatitis B, or hepatitis C infection [43,48]. In order to improve the activity in cancer patients, subsequent clinical trials conducted were based on the transfer of DCs pulsed with α -GalCer [45,46]. These trials again failed to display any noticeable anti-tumor effect. In a recent Phase I clinical trial, however, APCs were pulsed with α -GalCer in the presence of autologous V α 24 NKT cells, before transferring them back to the cancer patients. In short, the combined use of the intra-arterial infusion of activated V α 24 NKT cells and the submucosal injection of α -GalCer-pulsed APC induced significant antitumor immunity and had measurable beneficial clinical effects in the management of advanced head and neck squamous cell carcinoma [44].

Our recent work has demonstrated that α -GalCer-activated NKT cells enhance malaria-specific T cell responses and, more importantly, protective anti-malarial immunity elicited by various immunogens, including a recombinant adenovirus expressing a malarial antigen [49]. The adjuvant effects of α -GalCer in this model require CD1d molecules, V α 14 NKT cells, and IFN- γ (Fig. 1). However, two recent studies have shown that α -GalCer or its analogues can display their adjuvant effects on T cell responses elicited by a soluble protein, possibly through CD40–CD40L interaction, as well as by cytokines such as type I interferon and IFN- γ [40,50]. It was also shown that these interactions may ultimately lead to the full maturation of dendritic cells, and are therefore responsible for mediating the adjuvant activity of glycolipids [40,41,50,51]. More recently, a number of studies have reported on the adjuvant effect displayed by α -GalCer and α -C-GalCer on vaccines against viruses and cancers in a mouse model [52–56]. Interestingly, a very recent study has shown that non-glycosidic analogues of α -GalCer could activate iNKT cells resulting in dendritic cell maturation and the priming of antigen-specific T and B cells, thereby acting as a vaccine adjuvant [57].

1.3. The discovery of a lead clinical compound, 7DW8-5, and its adjuvant activity

In an attempt to search for a more potent glycolipid, we synthesized a compound library of 25 glycolipids analogous to α -GalCer and evaluated their biological activity. The evaluation process included testing the ability of each glycolipid to stimulate human NKT cell lines and secrete cytokines including IFN- γ , as well as their ability to stimulate autologous DCs and secrete cytokines including IL-12. After selecting 5 glycolipids which displayed strong biological activity in *in vitro* assays, we carefully assessed the binding affinity of selected glycolipids to the human CD1d molecule, as well as to the invariant TCR (invTCR) of human NKT cells *in vitro*. The binding affinity of selected compounds to invTCR has been shown to have a strong correlation with its biological activity. Thus, after evaluating the

biological activity of these glycolipids, we identified 7DW8-5 as a lead compound for further adjuvant testing [58].

After selecting 7DW8-5 as a lead compound, we compared its adjuvant effect with that of α -GalCer against various HIV vaccine platforms, namely Ad5-p24 and DNA-p24. For this purpose, different doses of each compound were co-administered intramuscularly with a sub-optimal dose of either Ad5-p24 or DNA-p24. Two weeks after a single immunizing dose of Ad5-p24 co-administered with each glycolipid, 7DW8-5 was shown to enhance the most the level of p24-specific CD8⁺, as well as CD4⁺ T cell responses that secrete IFN- γ , as determined by ELISpot assay [58]. Similarly, when mice were primed with a DNA-p24 vaccine plus either 7DW8-5 or α -GalCer, and then boosted with the DNA-p24 vaccine alone, 7DW8-5 displayed a stronger adjuvant effect than α -GalCer, eliciting significantly higher p24-specific CD8⁺ T cell and humoral responses. Next, in order to test whether 7DW8-5 displays its adjuvant effect on “protective” immunity induced by vaccines, we used Ad5 expressing a malarial antigen, Ad5-PyCS, as a vaccine platform. Two weeks after vaccinating mice with Ad5-PyCS together with or without a glycolipid, we challenged the vaccinated mice with live rodent malaria parasites. Then, we determined the amounts of parasite loads in the liver of infected mice. Non-immunized mice were used as a control. Through this challenge experiment, we confirmed that 7DW8-5 displays a more potent adjuvant effect than α -GalCer, leading to a significantly higher degree of “protection” against malaria in mice [58]. Finally, to ensure that the adjuvant effect of 7DW8-5 was mediated by CD1d molecules, we immunized mice lacking CD1d, as well as wild-type mice, with Ad5-p24 co-administered with 7DW8-5, and measured the level of p24-specific T cell responses. Not surprisingly, the adjuvant effect of 7DW8-5 was totally abolished in mice lacking CD1d molecules, confirming the CD1d mechanism-based adjuvant effect of 7DW8-5. These studies have led us to select 7DW8-5 as the lead compound for further clinical development [58].

2. Preclinical development of 7DW8-5 as an adjuvant for a candidate malaria vaccine

2.1. NMRC-M3V-Ad-PfCA candidate malaria vaccine

AdPfCA is a non-replicating adenovirus serotype 5 based malaria vaccine candidate developed by the US Military Malaria Vaccine Program (USMMVP), GenVec, Inc. and USAID, and expresses two *Plasmodium falciparum* antigens—circumsporozoite protein (CSP) and apical membrane antigen-1 (AMA1) [59,60]. The CSP is expressed by pre-erythrocytic stages, whereas AMA1 antigen is expressed by both pre-erythrocytic and erythrocytic stages [61,62], of the *Plasmodium* parasite life cycle. Thus, a combined immune response directed against these two antigens has the potential to reduce the number of malaria infections in healthy individuals by killing sporozoites and liver stage parasites and also to reduce the severity of malaria disease in those that do become infected by killing blood stage parasites. Based upon the ability of 7DW8-5 to adjuvate Ad5- and DNA-based vaccines as described above, we hypothesized that 7DW8-5 coadministered with AdPfCA would boost the immune response against both CSP and AMA1 in comparison to AdPfCA alone, thus affording a potentially better clinical outcome against malaria.

2.2. Go/No-Go studies

To test this hypothesis, we designed a series of critical “Go/No-Go” studies in the murine model that would serve as a gatekeeper to advancing this antigen–adjuvant combination into a Phase 1 proof-of-concept clinical trial. The key study was developed to determine whether an optimal dose of 7DW8-5 could significantly augment specific immune responses elicited by varying doses of AdPfCA. The predefined criterion for adjuvant efficacy was a two-fold increase in cell-mediated immune responses or a three-fold increase in humoral immune responses induced by immunization with AdPfCA in the presence of 7DW8-5 compared to those induced by AdPfCA immunization alone.

In summary, six BALB/c mice per group were immunized intramuscularly with AdPfCA doses ranging from 2×10^6 to 2×10^{10} particle units (pu) with or without an optimal dose of 7DW8-5 identified in previous murine studies [58]. Spleno-cytes and sera were collected from each group of mice two weeks post-immunization and used to determine the number of IFN γ -secreting cells (by ELISpot) and level of antibody titers (by ELISA) induced against CSP and AMA1. Results demonstrated that the presence of 7DW8-5 during immunization provided a greater than two-fold enhancement of cell-mediated immune responses against both CSP and AMA1 at AdPfCA doses of 10^7 , 10^8 , and 10^9 pu (Fig. 2). Notably, in some cases, the cell-mediated immune response levels induced by immunization with AdPfCA alone were surpassed using 100- to 1000-fold lower dosages of AdPfCA when co-administered with 7DW8-5. For example, the AMA1 specific response observed after mice were immunized with 10^7 pu of AdPfCA in combination with 7DW8-5 was almost two-fold higher than the AMA1 specific response observed with up to 10^{10} pu of AdPfCA alone. These data indicate the potential for a significant dose-sparing adjuvant effect when 7DW8-5 is used in combination with AdPfCA, which would provide substantial cost reduction in widespread vaccine manufacture. A dose-sparing effect would also reduce the volume of vaccine needed per injection to achieve the same efficacy as the same vaccine administered without adjuvant. This opens the door to combining multiple vaccine strategies, usually prohibited by maximum volume limitations, into one single injection that could provide greater and broader protection. Also notably, the adjuvanted vaccine achieved ELISpot responses that were higher than any achieved by the unadjuvanted vaccine. Even more significant than the dose-sparing effect, the ability to achieve absolute increases in the magnitude of immune responses is promising for future applications. While a trend towards increased humoral immune responses against CSP and AMA1 was also noted, their level of enhancement did not meet our pre-defined criteria. However, the degree of enhancement achieved in antibody responses will remain an important consideration as future testing occurs. Refinement in non-human primates of the optimal AdPfCA and 7DW8-5 doses determined in mice and further evaluation of any potential safety and toxicity concerns will be critical before moving into a Phase 1 clinical trial in healthy human volunteers.

3. Practical considerations in the clinical development of glycolipid adjuvants

3.1. Manufacturing

Unlike laboratory reagents for use in small animal studies, all biologic substances must be manufactured under Good Manufacturing Practice (GMP) conditions (Code of Federal Regulations Title 21, Part 211, April 2009) before use in humans. This requires that compound synthesis be reproducible and scalable at large quantities. One of the practical advantages of glycolipids is their straightforward synthesis pathway from chemical compounds that are readily available at relatively low cost. In order for glycolipids to function as effective adjuvants that provide dose-sparing of vaccines against infectious diseases with high prevalence in the developing world, low cost and ease of manufacturing are crucial.

3.2. Formulation

Glycolipids are amphipathic molecules consisting of a hydrophilic sugar head, which engages the invariant T cell receptor on NKT cells, and two highly hydrophobic lipid tails, which lie in the CD1d binding groove [63]. In small animal studies, they are often solubilized in dimethyl sulfoxide (DMSO), which is toxic to cells in large quantities and therefore unacceptable for use in clinical product formulation. The majority of clinical trials of glycolipids to date have employed pulsing autologous dendritic cells with α -GalCer *ex vivo* [45,64–67], thus minimizing formulation issues of the compound alone. Only three clinical trials to date have involved direct administration of a glycolipid compound to humans [43,47,48]. In all studies, the active formulation was composed of 0.2 mg lyophilized α -GalCer vialled with sucrose, L-histidine, and polysorbate 20, a surfactant used in several commercial products as a detergent and emulsifier (Kirin Pharmaceutical Co. Ltd., Gunma, Japan). Yet even with the use of polysorbate, the concentration of α -GalCer was 0.2 mg/mL when reconstituted with sterile water. Thus, the volume of administration required to achieve the target doses necessitated IV administration of each dose.

While this approach is suitable for treating patients with cancer or hepatitis, it is not practical for administering vaccines for the prevention or treatment of infectious diseases on a global scale. Vaccines are typically administered intramuscularly (IM), with a maximum tolerable injection volume of 1 mL in humans. Administration into the subcutaneous space may allow for slightly greater volumes, but with a maximum of 3 mL depending upon the injection site [68]. Because glycolipids provide their optimal adjuvant effect when co-administered at the same time and location as the target vaccine [36], the total injection volume must allow for both vaccine antigen and adjuvant together. Therefore, the application of glycolipids as vaccine adjuvants in humans necessitates improved formulation methods to achieve a high enough concentration to provide an effective adjuvant dose in a small enough volume for injection with vaccine. Several newer methods to achieve higher concentration of biologic compounds are being developed, including hydrogels, liposomes, and micro-spheres [69,70].

3.3. Safety

Giaccone et al. reported administering IV α -GalCer to patients with solid tumors in doses of 50–4800 $\mu\text{g}/\text{m}^2$, on days 1, 8, and 15 of a 4-weekly cycle, for up to 6 cycles [43]. In this study, α -GalCer was well-tolerated at a wide range of doses, with no drug-related serious adverse events, and no evidence of hepatotoxicity, which had been previously reported in the mice exposed to IV α -GalCer [71,72]. Similarly, Veldt et al. observed that IV administration of 0.1 $\mu\text{g}/\text{kg}$, 1 $\mu\text{g}/\text{kg}$ or 10 $\mu\text{g}/\text{kg}$ (versus placebo) was well tolerated in patients with chronic hepatitis C infection. Patients experienced a transient decrease in circulating NKT cell levels, but only the patient with the highest pre-treatment NKT cell level showed signs of immune activation and a subsequent 1.3 log decrease in HCV-RNA levels. Woltman et al. administered 0.1 $\mu\text{g}/\text{kg}$, 1 $\mu\text{g}/\text{kg}$ or 10 $\mu\text{g}/\text{kg}$ IV α -GalCer to patients with chronic hepatitis B at weeks 0, 4 and 8 [47]. In contrast to the previous studies, subjects reported a high frequency of fever and headache (78% and 63% respectively), likely due to systemic cytokine secretion resulting from generalized immune activation. One of eight patients in the mid dose group and three of eight patients in the high dose group did not complete the therapeutic regimen due to side effects. The authors concluded that the poor tolerability made α -GalCer unsuitable as an alternative monotherapy to current treatment regimens for hepatitis B. This trial underscores the need to characterize the level of immune activation and cytokine response to each glycolipid in stringent preclinical animal models. An ideal glycolipid adjuvant would provide local immune activation without a strong systemic cytokine release in order to minimize the risk-benefit ratio.

3.4. Dosing

Determining the dose of glycolipid required to provide the optimal adjuvant effect of a particular vaccine in humans is a non-trivial exercise which depends upon a number of factors. For many small molecule compounds, the effectiveness increases with increasing dose, so the maximum dose is determined by the level at which toxicity and safety considerations outweigh the benefits provided by the drug, the so-called maximum tolerated dose (MTD). The dose effect of glycolipids as vaccine adjuvants is significantly different because of their mechanism of action. While too low a dose will not provide sufficient NKT cell stimulation to recruit and activate secondary immune cells at the site of vaccination, high concentrations of glycolipid drive the NKT cells to an anergic state, also abrogating the adjuvant effect. In addition, various glycolipids may have differing dose-response curves. Fig. 3 depicts the differing dose curves of the adjuvant effect of 7DW8-5 on the IFN- γ ELISpot response to an adenovirus serotype 5 based vaccine encoding the CS antigen [58]. The maximum effective dose is 0.1 μg of 7DW8-5, which decreases at a higher dose of 1 μg . Thus, determining the optimal dose of glycolipid adjuvant for each vaccine is critical in order to optimize immunogenicity.

In addition, the optimal amount of glycolipid required may vary depending upon the type of vaccine used. For example, viral vectors may require a lower level of NKT cell stimulation than DNA-based vaccines, given the inherently larger inflammatory response to viral vectors, which contain foreign antigens independent of the vaccine insert. Furthermore, the ratio of the optimal glycolipid dose to vaccine dose may play a role in the dose response,

and may vary by antigen. Studies to answer these critical questions are in progress in our laboratory.

Finally, many small molecule drugs are developed in small animals and then translated to doses in humans by administering an equivalent number of milligrams per kilogram, on a linear weight-based scale. The immunologic activity of biologic compounds such as glycolipids or cytokine adjuvants is complex and often more localized than the small molecule drug compounds, and therefore not predictable using linear weight-based scaling. This critical limitation necessitates dose-finding studies in larger animal species, such as non-human primates in order to determine effective doses for clinical testing.

3.5. Species selection

The NKT cell response to glycolipids in rhesus macaques has been studied, but to a limited extent. Like humans, rhesus macaques possess V α 24 T cell receptor positive T cells. The amino acid sequence of the V–J junction of the V α TCR is 93% homologous to humans, and the CD1d alpha1–alpha2 domains of both species have 95.6% homology [73], which would imply that the rhesus macaque model is predictive of glycolipid responses in humans. However, Gansvud et al. subsequently demonstrated that rhesus NKT cells in the spleen have increased CD3⁺CD8⁺ and CD3⁺CD56⁺ coexpression, and display a more Th2 skewed phenotype in comparison to peripheral blood rhesus NKT cells [74]. As described above, the generation of an effective adjuvant response relies primarily on eliciting a strong IFN- γ response, a Th1 response, whereas Th2-skewed NKT cells are involved in the protection from autoimmune diseases such as diabetes [75]. Given that the alteration of the glycolipid structure can shift the Th1/Th2 balance [24,26,76], it is critical to test each new compound in the desired target species and the desired target cell type for replication of the appropriate response before utilizing that species for safety, dosing or other preclinical studies.

4. Clinical trials and future development

4.1. Clinical testing of the safety and immunogenicity of 7DW8-5 as a vaccine adjuvant

Although the ability of glycolipids to act as vaccine adjuvants has been well established in the animal model, neither α -GalCer nor any compound derivatives have been tested in combination with vaccines in clinical trials. In a Phase 1 clinical trial conducted by the USMMVP, one dose of the unadjuvanted vaccine elicited strong cellular immunogenicity as measured by *ex vivo* IFN- γ ELISpot assay [60]. In a follow-on clinical trial, in which NMRC-M3V-Ad-PfCA was given as a boost sixteen weeks after priming with three doses of DNA (also encoding PfCSP and PfAMA1), a significant proportion of the volunteers was sterilely protected against *P. falciparum* sporozoite challenge [59].

Glycolipid adjuvants offer the potential to augment this vaccine regimen for a number of reasons. First, glycolipids have been shown to effectively augment adenovirus based vaccines encoding a number of antigens in small animals, including NMRC-M3V-Ad-PfCA, as described earlier. Second, they preferentially boost cellular immunity, which may be particularly important for protection against malaria due to its complex intracellular lifecycle [77,78]. Third, in prior experiments in small animals, α -GalCer has been shown to assist in overcoming pre-existing anti-vector immunity to adenoviral vectored vaccines [49].

We therefore intend to evaluate the adjuvant effect of 7DW8-5 in healthy volunteers in a Phase 1 clinical trial by administering a single fixed dose of NMRC-M3V-Ad-PfCA with or without varying doses of 7DW8-5. As this will be the first administration of this adjuvant in humans, the primary objective will be the safety of the 7DW8-5/NMRC-M3V-Ad-PfCA combination. Secondary objectives will include the evaluation of adjuvant effect on the cellular and humoral immunogenicity of both CSP and AMA1, as well as the determination of the optimal dose of 7DW8-5 required for the adjuvant effect. As outlined in the previous section, given the potential of overdosing to induce NKT cell anergy, the determination of the optimal dose will be critical in pursuing further adjuvant studies with this compound. This clinical trial will be conducted after the critical animal studies to assess dosing, safety and toxicity are completed. If safety and immunogenicity results are promising, a further study is planned to measure the impact of 7DW8-5 on protection against malaria challenge. Of particular interest will be the ability of the glycolipid to counteract any negative effect of the pre-existing adenovirus 5 neutralizing antibodies on immunogenicity and protection.

4.2. Future applications

If the promising results demonstrating the strong adjuvant effect of glycolipid compounds on AdPfCA translate to humans, there are a number of potential future applications. Adenoviral vectors are being utilized for a number of candidate vaccines against malaria, HIV-1, and other pathogens [79,80]. It would be reasonable to predict that the adjuvant effect on adenoviral vaccines against differing antigens would be similar, although this hypothesis would need to be tested in separate clinical trials with each vaccine, as adjuvants must be licensed in combination with each individual antigen, rather than as standalone compounds. α -GalCer enhances the immunogenicity of a sindbis viral vector vaccine [49], and α -C-galactosylceramide, an α -GalCer analog, provides an adjuvant effect to influenza vaccine in mice [54]. It is important to determine whether glycolipid adjuvants can elicit similar effects on other viral vaccine vectors. These studies are underway in our laboratory.

In addition, α -GalCer has been shown to have an adjuvant effect on DNA-based vaccines in small animals [52]. Further clinical trials will be needed to assess whether this effect translates to humans. DNA vaccines are advantageous for a number of reasons, both immunologic and practical. DNA vaccines confer the ability to “prime” the response to viral vaccines, resulting both in the increased magnitude of the response, as well as a skewing toward a stronger CD4⁺ T cell response, which can act to provide help for more durable humoral and cellular immunogenicity [81-83]. On a practical level, DNA vaccines are easier to produce at lower cost than viral vaccines, and have a more favorable thermostability profile, which can be advantageous for mass production and distribution of vaccines to prevent infectious diseases in the developing world. Lastly, because DNA vaccines do not elicit anti-vector immunity, they can effectively continue boosting the immune response with multiple vaccinations (Y. Huang, personal communication), unlike the viral-vectored vaccines. Thus, boosting the immune response to DNA vaccines with glycolipid adjuvants could allow for therapeutic vaccine regimens against infectious diseases, cancer, or other diseases.

5. Conclusions

While glycolipid compounds have been studied as a potential cancer treatment in a number of trials to date, the use of glycolipid compounds as vaccine adjuvants has thus far been limited to research in small animals, with promising results to date. Clinical development of a novel glycolipid compound, 7DW8-5, is underway to determine whether it can provide an adjuvant effect on an adenoviral-based malaria vaccine in healthy human volunteers. Should this concept translate to humans, glycolipids offer a promising potential mechanism to augment the immunogenicity and efficacy of vaccines against a variety of infectious diseases at relatively low cost.

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Figure 1.

Role of NKT cells in adaptive immunity against malaria and HIV. In the context of CD1d molecules, a glycolipid can stimulate NKT cells through their invariant T cell receptor (invTCR). Upon activation, NKT cells rapidly secrete cytokines such as IFN- γ , and together with CD40–CD40L interaction, induce the activation and maturation of dendritic cells. Thus, the co-administration of a glycolipid (such as 7DW8-5) with vaccines expressing malaria or HIV antigens may be able to enhance the efficacy of the vaccines by augmenting the levels of antigen-specific T cell and humoral responses.

Figure 2.

7DW8-5 enhancement of cell-mediated immune response to AdPfCA. Groups of BALB/c mice (n=6) were immunized with varying doses of AdPfCA (adenovirus co-expressing *Plasmodium falciparum* CSP and AMA1) together with or without 0.1ug 7DW8-5. Splenocytes were collected two weeks after immunization to determine the relative number of IFN-γ-secreting peripheral blood mononuclear cells (PBMCs) by an ELISpot assay. Numbers in parentheses indicate the fold increase of CSP-specific (red) and AMA1-specific (blue) IFN-γ-secreting PBMCs after immunization with AdPfCA+7DW8-5 (circles) as compared to after immunization with AdPfCA alone (triangles).

Figure 3.

Dose effect of 7DW8-5 on a malaria vaccine in mice. Groups of BALB/c mice (n=5) were immunized with AdPyCS (adenovirus expressing a *Plasmodium yoelii* CS protein) together with indicated amounts of 7DW8-5. Splenocytes were collected two weeks after immunization to determine the relative number of IFN- γ -secreting CD8⁺ T cells by an ELISpot assay. Adapted from Li et al., 2010.