OBAT: Jurnal Riset Ilmu Farmasi dan Kesehatan
Volume 3, Nomor 3, Mei 2025
e-ISSN: 3031-0148, p-ISSN: 3031-013X, Hal. 09-27
DOI: https://doi.org/10.61132/obat.v3i3.1227
Available Online at: https://journal.arikesi.or.id/index.php/OBAT

Chemical Productions and Immunological Assessments of Cancer Vaccines According to A-Galactosylceramide and Ganglioside Antigen

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Abstract. *iNKT cells, sometimes known as the immune system's "Swiss Army knife," have become key components of cancer vaccination treatments. Glycolipids that activate iNKT cells, including α-galactosylceramide (αGalCer), have been used to create self-adjuvanting anti-tumor vaccinations and can boost the immune response to co-delivered cancer antigens. The chemicals synthesis of ganglioside antigens, specifically (Neu5Gc) GM3 and GM3 antigen, and conjugations to αGalCer, and packaging into liposome as effective platforms for their in vivo deliverying are the main topics of this work. In mouse and human cell experiments, liposome containing, (Neu5Gc) GM3-αGalCer, GM3-αGalCer, and equimolar quantities of conjugates have thoroughly described and their capacities to activate iNKT cell has verified ex vivo. All subclasses of IgG antibodies were produced as a result of the candidates' potential to generate both TH1 and TH2 cytokines, according to in vivo immunization tests. Interestingly, this study shows serum antibody produced against the TACA were cross-reactive, both when they were produced separately and together. If tumor-selective antigens is selected, the ensuing antibodies response can be wider than expected, which has implications for future vaccine formulations.*

Keywords*: α-galactosylceramide and ganglioside antigen, cancer, chemical productions of cancer vaccines, immunological assessments of cancer vaccines.*

1. INTRODUCTION

Since the discovery of iNKT cells in the beginning of the 1990's and the first reports on their anti-tumor activity (Kawano et al., 1998; Lantz & Bendelac, 1994), cancer immunotherapy has identified iNKT cells as its desired target (Bedard et al., 2017; King et al., 2018; Zhang et al., 2019). Thus, it has been identified that α-galactosylceramide (αGalCer) is the standard iNKT agonist, which unlike typical T cell, recognize glycolipid bound to nonpolymorphic MHC class I like molecule, CD1d (Brigl & Brenner, 2004). In APCs, αGalCer directly targets to CD1d to execute its activity. The active CD1d /αGalCer/ TCR ternary complexes, where the crystal structure of human and mouse were determined in 2005, is formed following the binding of iNKT -T cells receptors to the CD1d and αGalCer cell surface complexes (Borg et al., 2007; Koch et al., 2005; Zajonc et al., 2005). In this way, the sugars moiety is fixed tightly by the intermuseum hydrogen bond step by step and extends out of the lipid binding groove surface and can be recognized by the TCR of iNKT cell. The binding between the two partners is controlled by helix interaction of α1 CD1d with TCR CDR. Most of these regions interact with 4-OH and 3-OH of galactos and also form hydrogeninc bonds with3′-OH of the phytosphingosine and 2-OH of the

Received: February 09, 2025; Revised: February 25, 2025; Accepted: March 12, 2025; Online Available: March 14, 2025

sugars moiety the chain where present (Banchet-Cadeddu et al., 2011; Girardi & Zajonc, 2012). The stimulated iNKT cells further deliver a cytokine message by generating TH1, TH2 and TH17 activity Type, and redirections include NK cell transactivation, DC stimulation, both CD8+ and CD4+ T cell activation, and B cell maturations. Although it has potential, αGalCer as a single treatment for clinical therapy is currently not practical because the impact of cytokine antagonists and reduction of iNKT cells growth following recurrent administration of αGalCer.

Since there have been few clinical studies on αGalCer, more focus has been placed on developing αGalCer analogs with different iNKT cell activating properties and TH2/ TH1 differentiations, as well as more effective delivery systems like liposomes (Speir et al., 2017; Verbeke et al., 2019), glycoimmunology has shown interest in α's adjuvant potential. It is important to highlight, nevertheless, that throughout past few decade, the standard method for creating Immunizations based on carbohydrates, including those for contagious illnesses, possesses the straightforward conjugations of carbohydrates antigen on peptide and immunogenic proteins. These systems, will displayed after APCs are dissected by the trimming procedure, offer high affinity antibodies in addition to immunological diagnostics with T cell responsiveness. Although it has been demonstrated that TACA can also be used as protein/peptide-based conjugates to prepare cancer vaccines, they did not meet the required clinical effectiveness, much like known carrier proteins, so new techniques for the synthesis of carbohydrate-based cancer vaccines have been sought (Wilson & Danishefsky, 2013).

This leads to the application of αGalCer to TACA, which has been recognized as a new approach to creating a fully synthetic cancer vaccine. The covalent coupling of the Tn antigen with αGalCer to form SialylTn (sTn), along with the synthesis of αGal-Cer-Tn and αGalCer-sTn liposomes, resulted in robust and specific production of IgG antibodies against both anti-sTn and anti-Tn (Broecker et al., 2018; Yin et al., 2017). The most prevalent type of immunoglobulin is called IgG. The highest concentrations are seen in tissue fluids and blood. Every IgG molecule made up of the basic four-chain immunoglobulin structure because has two identical antigen-binding sites it is (Ali et al., 2024).

Here are a few TACA–αGalCer design examples: Recently, αGalCer conjugates of GM3 ganglioside have been added to the TACA family (Yin et al., 2021). The TACA class, ganglioside TACAs are sialylated glycosphingolipids and cancers of neuroectodermal. This makes it a good candidate for the development of cancer vaccines

and antibodies treatment (Heimburg-Molinaro et al., 2011; Padler-Karavani et al., 2011). Human cannot synthesize Neu5Gc glycoconjugates since they have a gene deficiency in CMP-Neu5Ac hydroxylase which is necessary to manufacture (Neu5Gc) GM3. CholR from meat and dairy products is thus considered inhuman and it is assumed that Neu5Gc is absorbed into human tumors (Bashir et al., 2020). With these presumptions in mind, we have been interested in synthesizing GM3 and (Neu5Gc) GM3 conjugates with αGalCer in order to design fully synthetic vaccine constructs that could be incorporated in to Liposomes which is a good system for delivering both adjuvant and antigen to APC.

We presents straight forwards and efficient chemicals synthesis of the desired conjugate 1 and 2 (Figure. 1) by incorporating advanced techniques from αGalCer and ganglioside chemistry. Additionally, we provide our (Neu5Gc) GM3–αGalCer and GM3–αGalCer constructions' liposomal formulation and immunological assessment, both in vitro and in vivo.

2. RESULT AND DISCUSSIONS

Synthesis of GM3–αGalCer 1 and (Neu5Gc) GM3–αGalCer 2 chemically

Both TACA-αGalCer complexes were synthesized using GM3, NHS ester, and Neu5GcGM3 ganglioside, compounds 3 and 4, respectively, along with the correct amine-substituted αGalCer 5 (Figure 1). αGalCer 5 has a handle for derivatization and a six-carbon amino linker C6 to preserve adjuvanticity. It has been found that the ganglioside TACA include both an ethanolamine linker at the anomeric position as well as a further elongation with the N-succinimidyl glutarate chain. Tubbs et al previously described several methods for synthesizing αGalCer and its analogs. These approaches are tailored for the two main drawbacks of glycosylation, namely poor selectivity and low yield, to enable the addition of the comparatively inert ceramide. The most effective methods utilize Phytosphingosine protected by azide for post-glycosylation acylation, facilitating the incorporation of the fatty acid chain subsequent to glycosylation (Banchet-Cadeddu et al., 2011; Du & Gervay-Hague, 2005; Figueroa-Pérez & Schmidt, 2000).

Nonetheless, methods that directly react with acylated phytosphingosine typically exhibit insufficient α:β selectivity and low reaction yields (Cheng et al., 2019; Ma et al., 2020; Xia et al., 2006), which required the presence of The phytosphingosine diol is temporarily protected by (benzoyl) esters (Cavallari et al., 2014; J.-J. Park et al., 2008). Benzyl ether groups were introduced into the glycolipid to facilitate reactivity and synthetically versatility while the acylated phytosphingosine moiety was designed for

direct glycosylation during the glycolipid assembly. The aim of glycosylating Lactosyl diol 10 protected by benzyl ether and thioglycoside sialyl donors 8 and 9 was to achieve a suitable yield and stereoselective synthesis of the trisaccharide template needed for the synthesis of ganglioside TACA, (Neu5Gc) GM3, and GM3. Electrophilic Eight and nine Sialyl donors were prepared as derivatives of C-2 benzyl ester because the C-2 methyl ester analogues were less readily deprotected to form the final conjugates.

Synthesis of Linker Equipped αGalCer 5

N-Acetyl and N-fatty acylated phytosphingosine moieties were then synthesized to initiate the synthesis of αGalCer 5. In two processes, compound 11 was formed by the selective tritylation of primary OH group by trityl chloride/DMAP/pyridine at 80°C after treating phytosphingosine with N – (hexacosanoyloxy) succinimide and Et3N in THF and heating. When treating the acylated phytosphingosine scaffold with benzyl bromide and NaH in DMF, we were able to perform the benzyl protection reaction to both of the free hydroxyl groups thus synthesizing the intermediate benzyl protected molecule 12 in 87 % yield and without formation of the N-benzylated impurity. To our knowledge, this report provides the first demonstration of incorporating the benzyl ether protecting group into acylated phytosphingosine while eliminating the necessity to employ azidephytosphingosine or additional ceramide scaffold modifications. In this case, there are three synthetic benefits: 2). Benzylation of the acylated scaffold directly does not require zbyt elaborate protocol of protection or deprotection of the functioning of the amine, 3) all of the protective groups are simple to eliminate in a single action., 4) beneficial electron-donating effect of the benzyl groups during glycosylation.

Deprotection of the trityl protecting group was accomplished by treatment with p-toluenesulfonic acid in MeOH/CH2Cl2 (-7,84%). C-6 alkylation of thioglycosides 13 was employed to construct the galactosides moiety, starting with the requisite acylated sphingosine 7(Magaud et al., 1998). Under basic conditions with 6-chlorohexyl 4-methylbenzenesulfonate present (Mori et al., 2010). The reaction produced a 74% yield of compound 14. The azides were introduced by nucleophilic substitution using NaN3 in DMF while it was heated to -15, 84%. At -20°C in THF/Et2O, a first attempt at glycosylation was conducted using thioglycosides 15 and benzylated ceramide 7 while TfOH/ NIS was present. The reaction produced a 77% yield of the desired product, 17 albeit as a 2:1 α: β mixture. Although the desired αGalCer scaffold was accessible through the process, a more effective glycosylation was looked into. For example, 15 was

hydrolyzed under standard conditions (NBS, H2O /acetone, 16, 84%) to yield its correspondings N-phenyl 2,2,2-trifluoroacetimidate donors (Yu & Tao, 2001).

This was followed by a reaction with 2,2,2-trifluoro-N-phenylacetimidoyl chloride in the present of Cs2CO3 (6, 85%). At -20°C and in Et2O /THF, TMSOTf-promote glycosylations with glycosyl donor 6 and acceptor 7 thankfully produced the desired products 17 in 72% and with full α-stereoselectivity. With just nine reaction steps and an 11% global yield, the developed strategy thus made it possible to easily and simply form a full protect αGalCer bearing important functionalizations handle (i.e., terminal azides). By reducing the azide through zinc treatment in an acidic environment, compound 17 was further derivatized, yielding αGalCer 5 in a 67% yield.

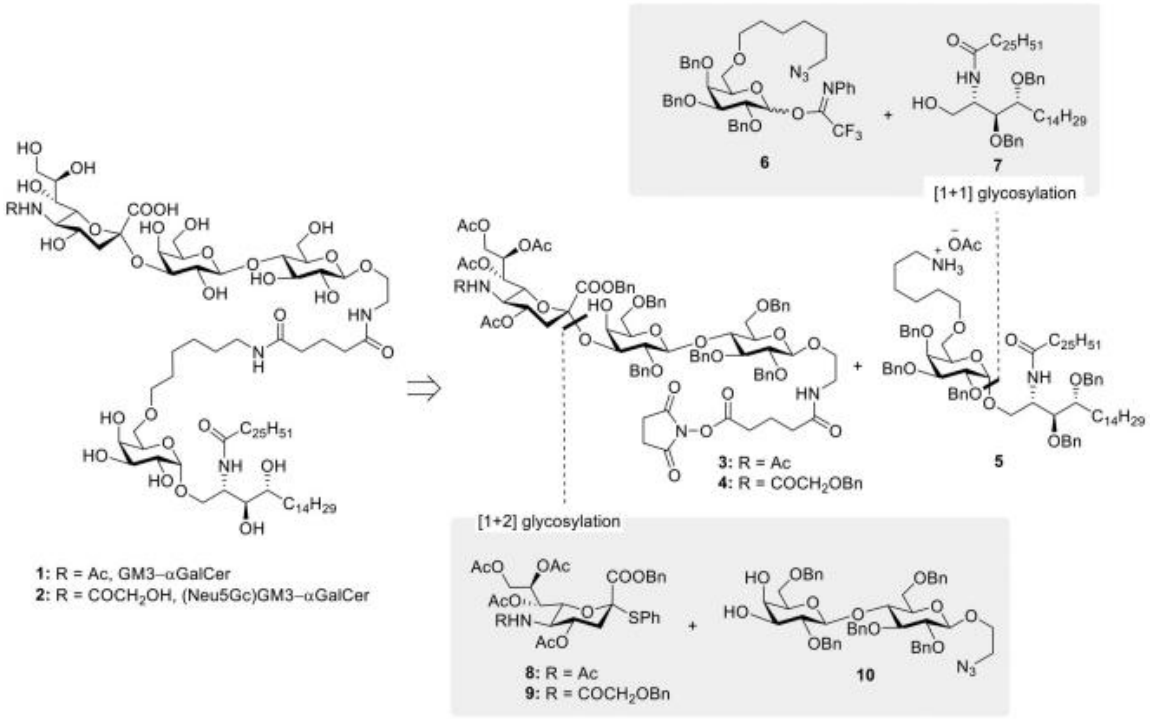

Figure 1. Worldwide plan for 1 and 2 synthesis.

TACA derivative 3–4 synthesis and conjugations to αGalCer

Linker equipped-lactose 10 (Yu & Tao, 2001) was easily synthesized in 7 reaction steps and in 32% global yield utilizing the method described in the literature to produce the necessary TACA–αGalCer derivative 3 and 4. The α-anomer, Four steps were also used to create sialyl donor 8 (38% global yield, ESI). The main reaction was the matching glycosyl chloride being substituted for thiophenol in a straightforward, SN2-like way.

Conversely, sialyl donors 9 was created using 8. The initial steps involved treating methanesulfonic acid under reflux in order to achieve deacetylation and amide hydrolysis. O-benzylglycolic acid succinimidyl ester, which is readily synthesized, was then applied to the crude combination under simple conditions. The final product underwent three procedures of acetylation under standard circumstances (Ac2O, pyridine) to yield sialyl donor 9 in 56%. In CH2Cl2/CH3CN at -40 °C and-78 °C, sialyl donor eight and nine interacted with lactose acceptors ten in the existence of AgOTf /IBr, respectively (Meijer & Ellervik, 2002).

Complete stereo- and regioselectivity was demonstrated by the high yields of the intended products 18 and 19 produced by both glycosylations (85% and 71%, respectively). The proposed techniques reduce the numbers of glycosylations stages to one by using a lactose building block that was suitably protected, unlike other investigations that used more complicated approaches (Yin et al., 2021). Furthermore, both glycosylation procedures produced excellent yields, which reduced the need for post-glycosylation modifications even when the sialic acid donor had inherent acetamide functionality. The azide moiety was smoothly and selectively reduced after zinc was applied to compounds 18 and 19 in an acidic environment. Their process then employed the easily generated disuccinimidyl glutarate(Marsden et al., 2009).

With yields of 79% and 67%, respectively, N-hydroxyl succinimide derivative 3 and 4 are generated in the current state of Et3N. It was eventually discovered that reversing the reaction's order produced a cleaner reaction and a better yield, leading to the isolations of compound 1 and 2. The deprotection was first conducted under Zemplén conditions (MeONa, MeOH) by removing the acetyl ester group). Next, hydrogenolysis (H2, 5% Pd/C) was used to eliminate the benzyl ester and benzyl ether groups.

Liposome Formulations

Formulations resembling liposomes were employed for conjugates 1 and 2. In vivo, the immune response can also be influenced by the multivalent presentation of (Neu5Gc) GM3 and GM3 TACA. For instance, mice who receive smaller lipid vesicles (200 nm) by oral absorption or subcutaneous (sc) injection exhibit a change in reaction toward the generation of IFNγ, a characteristic TH1 response (Brewer et al., 1998; Schwendener, 2014). APC trafficking of the vesicles is thought to be the reason for the fluctuating activity.The liposomes consisted of 1,2-diastearoyl-sn-g The physicochemical properties of each liposomal formulation were measured using zeta potential analyses which

demonstrated negative surface charges across the groups I–IV, DLS that proved the monodispersity of particles (~200 nm) and PDI of each liposome. To quantify the recovery of each of the distinctive part, liposome contents were examined in more detail utilizing RP-HPLC-MS/MS analysis. The figured Liposomes I –V are compared to ensure that the same quantum of active component is used in the experiment.

Table 1. Composition of liposomes I–V

Compound(s)	Mol ratio	Formulations
(Neu5Gc)GM3–αGalCer (2)	58.2:38.9:2.9	DSPC:Chol:2
GM3–αGalCer (1)	58.2:38.9:2.9	DSPC:Chol:1
GM3 and αGalCer	56.2:38:2.9:2.9	DSPC:Chol:GM3:αGalCer
αGalCer	58.2:39.0:2.9	DSPC:Chol:αGalCer
Equimolar mix of (1) and (2)	56.2:38:2.9:2.9	DSPC:Chol:1:2

Immunological evaluation

Here, the liposome stimulate the activation of both human and murine DC, shown to induce the proliferation of iNKT in vitro. In a few hours after activation, iNKT cell secrete a large quantity of cytokines. A rise in IFN-γ occurs after the first increase in both IL-4 productions (Schwendener, 2014). In addition, iNKT cell constitutively have receptors that allow them to react instantly to certain cytokines, such IL-12 upon stimulation by activated APCs (Leite-de-Moraes et al., 1999).

First, liposome I–IV efficacy was tested using an ex vivo environment. To this end, mouse bone marrow-derived dendritic cell (BMDC) were co-cultured with iNKT cell following their stimulation with either I–IV or LPS. They were then incubated for 48 hours. When the liposomal formulations activated both the DC and iNKT cells, a drastic rise in both the IL-4 as well as IFN-γ levels was observed (Figure 2A and B). Moreover, DC pulsed with I–IV increased both IL-4 and IFN-γ in iNKT cell production compared to DC stimulated with LPS. This holds good for the idea that the exposure to immunity can be achieved by I–IV. We then sought confirmation on whether human iNKT cells might be activated with liposome formulations I–V ex vivo. T cells isolated from humans were cultured together with MUTZ3-derived human immature DCs, following their activation of maturity in the presence of I–V (Figure 3A–C and S7†).

No effect was observed for groups IV and V; however, for groups I–III that formed DC, CD1d expression enhancement was detected. Both CD8+ lenited cells and all developed DCs irrespective of the presence of liposomes expressed the markers HLA-DR,

CD209, CD1a, and CD83 at similar levels (Figure 3, panel A and S7†). Cells primed with III and to some extent I stained for the presence of the activation-marker consistently larger frequencies of iNKT cells than did samples primed with II, IV or V.

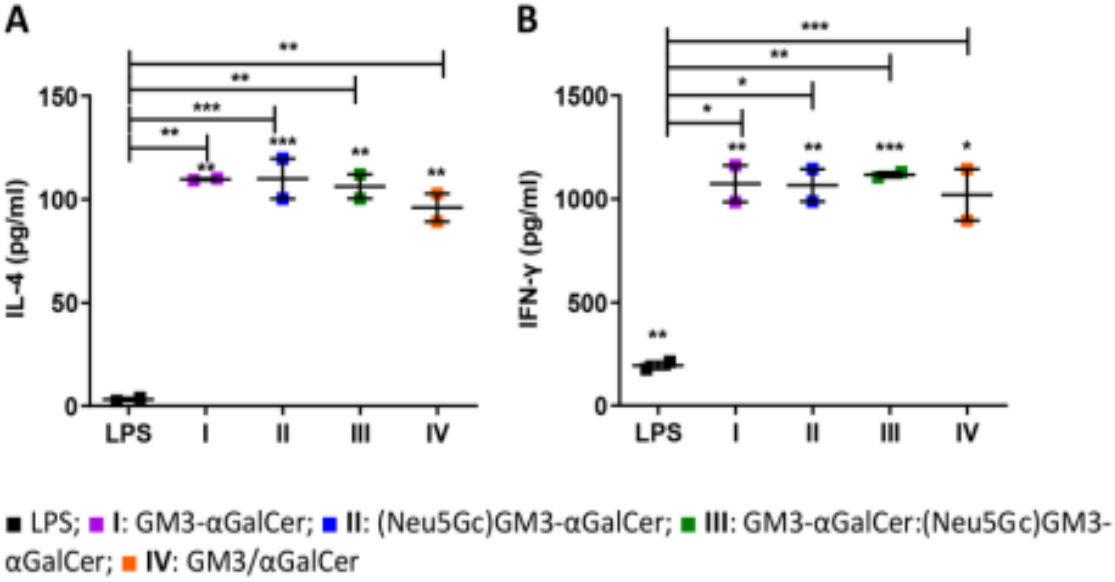

Figure 2. In co_culture supernatant, the vaccines candidate promoted ex vivo production of IL-4 and IFN-γ (A and B). Both 10 ng mL−1 I–IV and 100 ng mL−1 LPS were administered to BMDCs that were isolated from naïve mice. Isolated iNKT cell were then incorporated into the culture. The supernatant's cytokine secretion was quantified using a cytometric bead array.

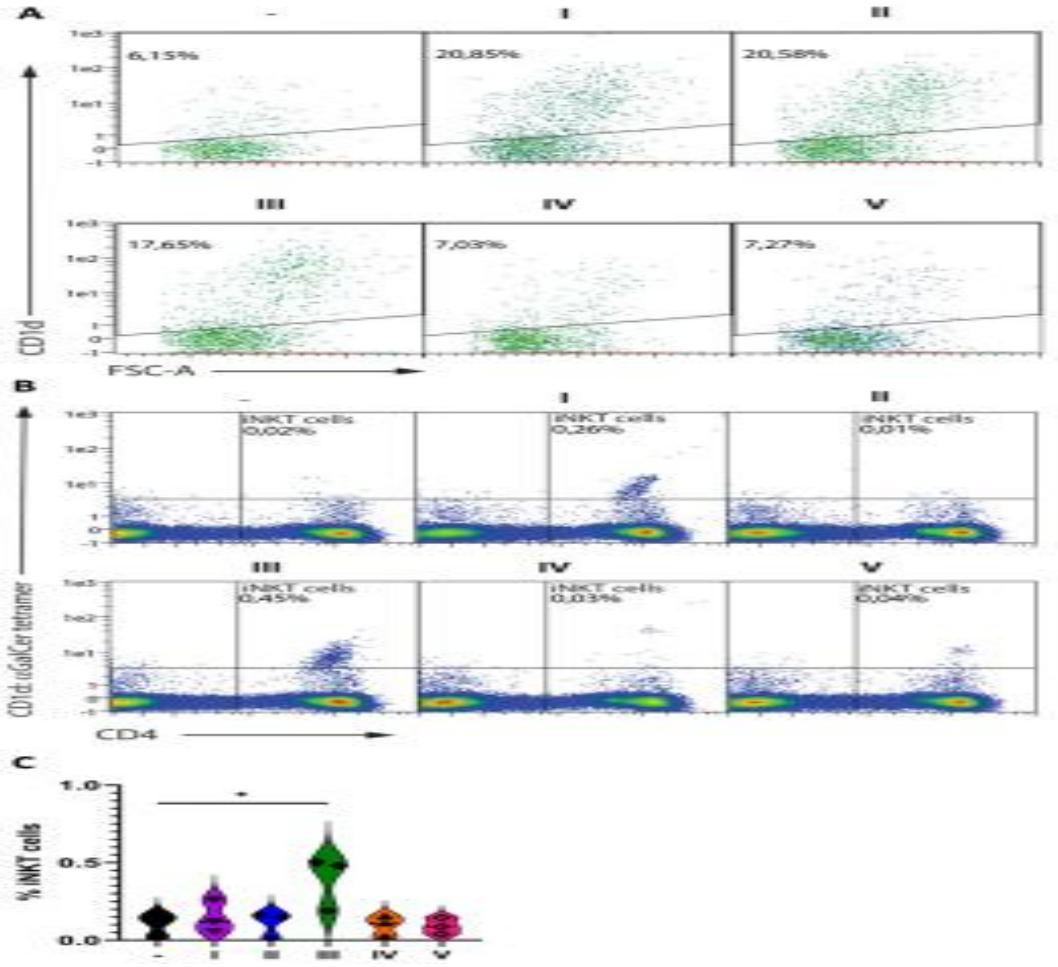

Figure 3. Human DC maturation and human iNKT cell activations (A, B, C). Human MUTZ-3-derived DC were primed either with or without the liposomal formulations I–V prior to flow cytometry analysis (A) or co-cultured with human CD3+ lymphocyte and then subjected to flow cytometry analysis for iNKT cell (B, C). Singlet and viable cell (based on staining with liveor dead yellow – Figure S6) were gated for all results. While C summarize the percentages of positive cell from three distinct tests employing cell from various donors, A, B display representable dot plot from three separate trial.

The Liposomes Cause in Vivo Cytokines, iNKT Activation, and iNKT Numbers.

Here, the liposome stimulate the activation of both human and murine DC, shown to induce the proliferation of iNKT in vitro. In a few hours after activation, iNKT cell secrete a large quantity of cytokines. A rise in IFN-γ occurs after the first increase in both IL-4 productions (Schwendener, 2014). In addition, iNKT cell constitutively have receptors that allow them to react instantly to certain cytokines, such IL-12 upon stimulation by activated APCs (Leite-de-Moraes et al., 1999). First, liposome I–IV efficacy was tested using an ex vivo environment. To this end, mouse bone marrow-

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Both CD8+ lenited cells and all developed DCs irrespective of the presence of liposomes expressed the markers HLA-DR, CD209, CD1a, and CD83 at similar levels (Figure 3, panel A and S7†). Cells primed with III and to some extent I stained for the presence of the activation-marker consistently larger frequencies of iNKT cells than did samples primed with II, IV or V. Intracellular IFN-γ levels and CD25 and CD69 were found on iNKT cells elevated in all mouse groups exposed to liposomes I–V, indicating that the activation signal pathway is experienced by a significant percentage of iNKT cells (Figure 4D–G). The percentage of splenic iNKT cells was not enhanced with the III, suggesting that III generates cytokines but does not promote activation and proliferation of iNKT cells as the other liposome formulations did 48 hours post-treatment. Thus, it is necessary to provide more detailed exploration of the essential nature of the interaction between immune-stimulating liposomes and iNKT cells.

Some interesting comparing of the iNKT cell expansion between the mouse in vivo system (Figure. 4) and human ex vivo system (Figure. 3) is presented here; while human iNKT cells expanded more in system I and III than that in system II, the mouse iNKT cells showed the opposite trend. Further, there were no differences in the unconjugated components (IV), αGalCer alone (V), and conjugates (I–III) activation efficiency. This implies that while αGalCer is the main determinant of mouse iNKT cell activation with these liposomal formulations, the activation of human iNKT cells is influenced by sugar antigens. Such a trend is supported by previous findings regarding differences in the TCR fine specificity of human and mouse iNKT cells that seem to correlate with differences in the activation and proliferation of these cells.

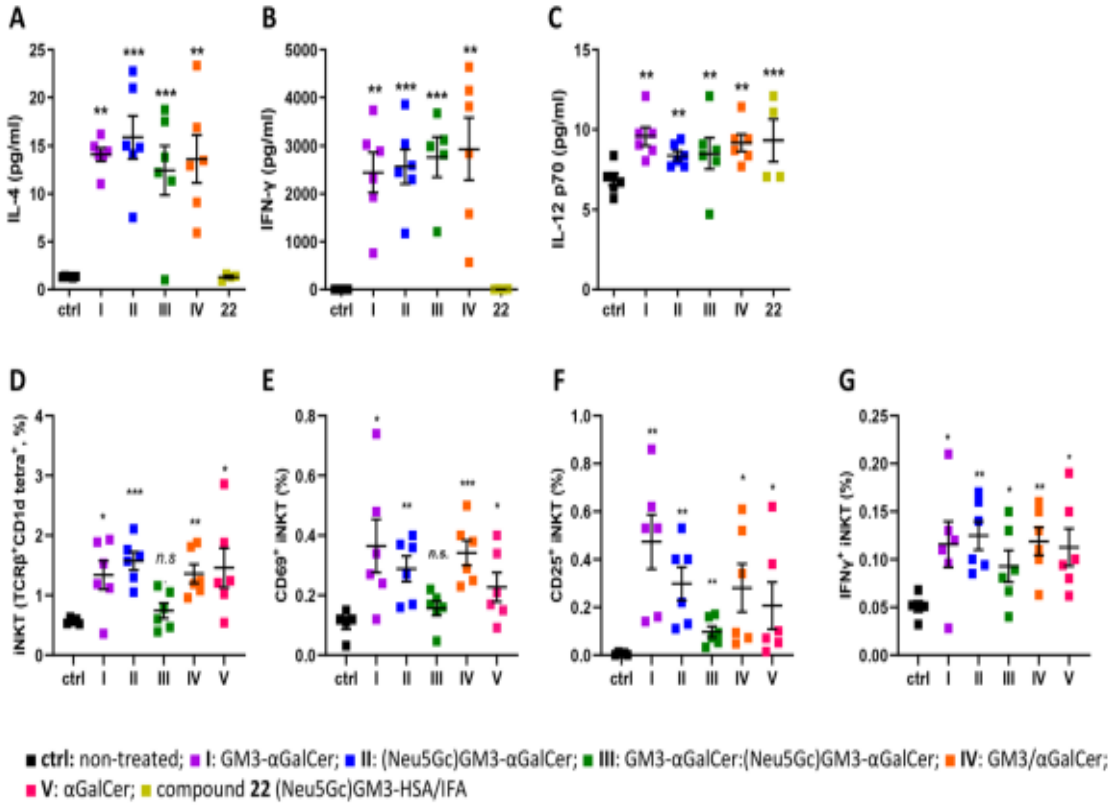

Figure 4. (A–C) Cytokine serum concentrations assessed by CBA 24 hours following the initial injection. (D–G) Flow cytometry was used to assess the production and activation of splenic iNKT cell produced by Liposomes I–V 48 hours following the initial injection. (E–G) The absolute numbers of splenic iNKT cell with the activated phenotype (CD69+, CD25+, IFN-γ +) and (D) the proportion of iNKT cells (TCRβint CD1d: αGalCer tetramer+) among total splenic cells.

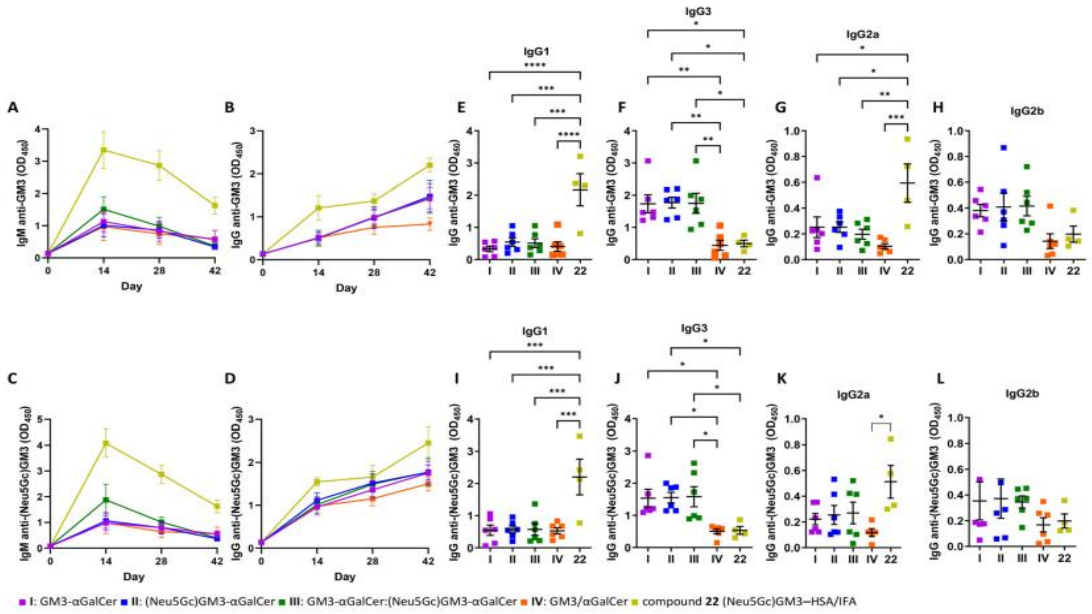

Figure 5. The production of antibodies and their isotype is stimulated by liposomal formulations I–IV and 22. The IgM and IgG reactions on days 14, 28, and 42 after GM3 (A and B) and (Neu5Gc) GM3 (C and D) vaccination. (E–H) and (Neu5Gc) GM3 (I–L) on day 42. After being vaccinated with sc every two weeks, the mice were put to death on day forty-two.

The Liposomes Induce Antibody Responses.

Serums were taken on days 0, 14, 28, and 42 after every two weeks (days 1, 15, 29), groups of six female C57BL/6 mice received sc vaccinations with liposomes I–V. (ESI, †Figure. S2). As a vaccination control, another group received an injection of emulsified glycoconjugate 22 in IFA. A tried-and-true method for getting around carbohydrate antigens' low immunogenicity is conjugation to protein carriers, which transmit glycopeptide epitopes to CD4+ T cells via MHC (Livingston, 1995). The immunization regimen for Group 22 was identical to that of the liposome I–IV treatment groups. Additionally, as a negative control, one set of mice was left untreated for the duration of the trial. First, the ability of the I–IV vaccination to stimulate it was studied if B cells might produce anti-GM3 or anti-(Neu5Gc) GM3 antibodies. As coating antigens (ESI), (Neu5Gc) GM3–HSA 22 or GM3–HSA 23 are used. ELISA was used to evaluate the IgM and IgG antibody responses. IgM class antibodies specific to carbohydrates were produced by mice inoculated with I–IV (Figure. 5A and C). Regarding IgG, every vaccination candidate effectively produced an anti-IgG reaction, which progressively grew throughout the course of the trial (Figure. 5B and D) Figure. S4 (ESI) displays the IgG and

IgM levels for the designated weeks. After the second and third vaccinations, IgM levels gradually rise while IgG levels gradually fall, suggesting that isotype swapping has been induced. Additionally, two weeks following the last injection, the IgG response that was produced by the vaccination procedure remained elevated.

Since IFA is known to affect the immune response and (Neu5Gc) GM3–HSA 22 is a glycoprotein, it triggers the helper T cell pathway, which causes mice inoculated with it to produce IgG and IgM (Livingston, 1995). IV may cause by the covalent bond between αGalCer and TACA in group I. Additionally, the IgM and IgG antibodies generated following immunization with formulations I and IV, which contained GM3 exclusively, cross-reacted with the (Neu5Gc)GM3–HSA coated ELISA microplates (Figure S4). The (Neu5Gc) GM3 antigen was delivered in formulations II and III, which produced antibodies in mice. These antibodies exhibited the same cross reactivity and were found to bind to the GM3 antigen in ELISA (Figure S4). The distribution of IgG antibody isotypes was also investigated. While TH1-type reactions promote isotype flipping to IgG3, TH2-type responses mostly result in the production of IgG1 in mice (Coffman et al., 1988). IgG1 and IgG2a levels were reduced in mice inoculated with liposome I–III, but anti-GM3 and anti-(Neu5Gc) GM3 IgG3 and IgG2b levels were greater (Figure 5E–L). The GM3 ganglioside inhibits the iNKT cells' TH2-like response, which helps to explain the same degree of switching23 found in a previous investigation (J. Park et al., 2008).

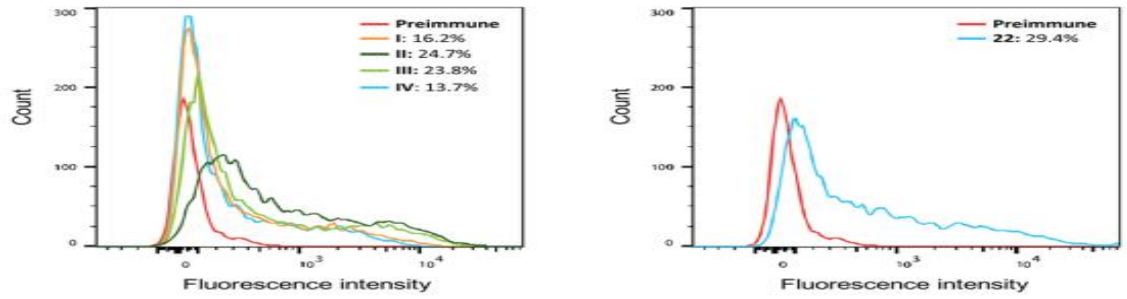

Figure 6. B16F10 cell binding to serum derived from vaccinated mice. Pooled sera from every mouse group were treated with B16F10 cells, and the binding was detected using flow cytometry and PE-conjugated anti-mouse IgG. The pre-immune serum of mice was utilized as the negative control prior to the initial vaccination.

To ascertain whether the antisera produced on day 42 could attach to the B16F10 cell line, which has been shown to express the GM3 antigen, flow cytometry was employed (Dorvignit et al., 2019). High titres of mice's serum following vaccinations showed positive staining on B16F10 cells in all vaccination candidates (Figure 6). Immunization

using liposomes I–III produced higher binding capabilities (positive cells: 16) than group IV, which contained αGalCer and non-conjugated GM3 ganglioside (13.7% positive cells). In the case of PGE2, the endogenously generated concentration was 0.01 μg/L (95% CI 0.01–0.02) following 24 hours of endotoxin infusion. This was raised to 2.0 μg/L (95% CI 1.7–2.2) following 48 hours, but then dropped to 0.1 μg/L (95% CI 0.06–0). Serum binding was 29.4% in mice and rabbits that received the protein conjugate vaccine (Broecker et al., 2018). Lastly, the capacity of antisera produced from immunization to stimulate the B16F10 cells' complement system was identified. A THP-1 cell line from acute monocytic leukemia that lacks the negative control was the expression of the GM3 and (Neu5Gc) GM3 antigens. On day 42, the antisera were initially treated with B16F10 cells. The number of dead cells was then assessed by treating the cells with complement protein. The B16F10 cells died as a result of the complement cascade on the surface being successfully triggered, as shown in ESI, Figure S5. Given this discovery, the vaccinated animals' antibodies have the ability to engage more selectively with B16F10 cells than with THP-1 cells that lack the expression of the particular ganglioside TACAs GM3 and (Neu5Gc)GM3.

3. CONCLUSIONS

On the other hand, an effective method for creating ganglioside-αGalCer conjugates has been created. Specifically, linker-functionalized αGalCer synthesis was done using a simple method which required a stereoselective glycosylation step based on the notorious inertness of the ceramide molecule. Likewise, the target GM3 and (Neu5Gc) ganglioside TACAs were anchored to αGalCer and synthesized in good yields. Unlike the methods which have been developed recently (Yin et al., 2021), the existing approach for the receipt of such. Thus, compounds under consideration need only one step of glycosylation and provide guarantee of α-selectivity. IgM and IgG antibodies generation was significantly and stably generated through the use of the formulations in the liposomes. Since the formulations of the tested it is commonly known that iNKT cells can assist B cells, and formulations lacked any helper peptide epitope in the initiation of an Ab response, because of affinity maturation and isotype switching, iNKT cells—rather than helper T cells—must be held responsible for the altered response to IgG in immunized mice. Furthermore, the antibodies triggered complement activation on B16F10 cell line surface-recognized and bound toward the B16F10 cell line. We also showed our formulations induced all subclasses of abdominal IgG antibodies since they produced

TH1- and TH2-associated cytokines including IFN-γ and IL-4 in mice. Moreover, the use of human derived DCs indicates that preparations of liposomes with ganglioside–αGalCer conjugates enhance maturation of human DCs and increase the expression of CD1d by about three-fold. On the other hand, the CD1d was not augmented by free GM3 with αGalCer. This is most likely due to the fact that human and mouse iNKT cells react differentially to stimulation by α-GalCer analogues with differing structures and affinities because of the fine specificities of the iNKT TCR–agonist–CD1d interaction (Wilson & Danishefsky, 2013).

In regard to the possibility of constructing the human environment in a mouse model, the outcomes in human DCs and iNKT cells appear promising. Further, it would be interesting to examine whether, and how, the further research on the role of sugar antigen processing in the human immune system and the inherent variations in iNKT cell activation caused by the various vaccine designs, as observed in human and murine DC cells, is advantageous. Based on the given data, systems for human testing remain indispensable for the translation of glycolipid cancer vaccines that target iNKT altogether These outcomes demonstrate how well the synthetic structures may control the immune response and offers fresh opportunities for developing other kinds of vaccines that can be enriched with components that could be used to shift the treatment approach towards either immune activation or suppression.

Serum antibodies formed against both of the TACAs, were cross reactive with each other and with GM3 and (Neu5Gc) GM3. Since most anti-glycan antibodies are expected to selectively recognize their target antigen, cross reactivity has been found for of the 80 different glycans (and glycoproteins), 27 have anti-glycan antibodies.). That the carbohydrate epitopes of the GM3 and (Neu5Gc) GM3 TACAs are shown and, therefore, are potentially immunogenic would not be totally surprising when the patterns of reactivity involving antibodies against GM3 or anti-(Neu5Gc) GM3 are considered (Manimala et al., 2007). So, despite high tumor selectivity of TACAs epitopes if antibody responses are broader than expected this finding is important to consider in the further development of carbohydrate-based cancer vaccines.

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