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Development and Functional Analysis of Th2-Biased Lipid-Modified CD1d Ligand

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Abstract

CD1d, one of the lipid antigen-presenting proteins, binds to a glycolipid ligand and forms a CD1d-ligand complex, which is recognized by NKT cells and induces the secretion of various cytokines including Th1 and Th2 cytokines. The cytokines are known to control immune responses, Th1 cytokines (e.g. IFN-γ) are involved in cell-mediated immunity such as tumor clearance and protection against infection, and Th2 cytokines (e.g. IL-4) are associated with humoral immunity such as allergies and promotion of antibody production. Recent studies revealed that the balance of the cytokines released by NKT cells depends on the CD1d ligand structures. α-GalCer (KRN7000) is a representative ligand and has a potent activity to induce both Th1 and Th2 cytokines. On the other hand, OCH is known as a Th2-selective CD1d ligand, and several clinical trials on using the ligand in patients with multiple sclerosis and Crohn's disease are ongoing. However, few studies of potent Th2-selective CD1d ligands have been reported compared with those of Th1-selective ligands, and the detailed mechanism of cytokine balance regulation remains unclear. Therefore, the development of potent Th2-selective ligands and elucidation of their biasing mechanism are required. In this article, we review the reported Th2-biased CD1d ligands and the cellular imaging with Th2-biased lipid-modified CD1d ligands for understanding Th1/Th2 selectivity.

Keywords

Lipid antigen
CD1d
α-GalCer
Lipid modification
Th1/Th2

1. Introduction

The antigen-presenting protein CD1d is found on antigen-presenting cells such as dendritic cells, among other cells involved in immune responses, and it binds to glycolipid ligands to form a complex. This complex is recognized by T-cell antigen receptors (TCR) on

the surface of natural killer T (NKT) cells and induces various cytokines, including Th1 cytokines such as IFN-γ, and Th2 cytokines such as IL-4. These cytokines are involved in different immune responses, Th1 cytokines are known to be involved in cellular immunity such as tumor elimination and infection defense, while Th2 cytokines are known to be involved in humoral immunity such as allergic inflammation and promotion of antibody production. Previous studies have shown that the induction and selectivity of cytokines released by NKT cells are influenced by the structure of CD1d ligands, and the creation of ligands that can regulate the balance of immune responses is desired.

A representative CD1d ligand known as α-GalCer (KRN7000) [1], a glycolipid derived from sponges, is a strong inducer of both Th1 and Th2 cytokines. As shown in **Figure 1**, the α-GalCer derivative developed by Wong *et al.* (**1 in Figure 1A**) [2] has an aryl group at the end of the acyl chain and has been reported to show Th1-selective cytokine induction properties. On the other hand, OCH [3] is known as a Th2-selective CD1d ligand and is currently being developed as a therapeutic agent for multiple sclerosis and Crohn's disease. However, compared to Th1-selective ligands, there are few reports of highly active Th2-selective ligands and limited knowledge of their regulation of cytokine balance. Therefore, the creation of highly active Th2-selective ligands and the elucidation of the mechanisms of their selective expression are important research topics.

Several groups have so far carried out the structural transformation of α-GalCer and developed lipid-modified α-GalCer derivatives with various selectivity. In this article, we review the α-GalCer derivatives as Th2-selective CD1d ligands reported so far and the mechanisms of Th1/Th2 selectivity expression, and introduce our recent findings.

2. Th2-selective CD1d ligand

A representative Th2-selective ligand is the aforementioned OCH, a derivative of α-GalCer with shorter sphingosine and acyl chains, developed by Yamamura *et al.* and undergoing clinical studies. Goff *et al*. have reported ligands with acyl chains (**2 in Figure 1A**) [4], and a ligand with a shorter acyl chain compared to α-GalCer (**3 in Figure 1A**) [5] was also found by Yu *et al.* Both have been reported to have Th2-selective cytokine-inducing activity. However, while shortening the length of the acyl chain improves Th2 selectivity, it is known that the binding affinity to CD1d decreases and the cytokine-inducing activity is significantly reduced.

As examples of other structural transformations, ligands in which a dialkylamino group is introduced into the acyl chain (**4 in Figure 1A**) [6], or the amide group is converted to a 1,2,3-triazole group (**5 in Figure 1A**) [7], or ester group (**6 in Figure 1A**) [8], have also been shown to induce Th2-type cytokine production.

In addition to the conversion of the ceramide moiety, Th2-selective CD1d ligands with modification of the 6-position of galactose have also been developed, with Jervis *et al.* reporting a triazole (**7 in Figure 1A**) [9], and Hu *et al.* introducing a p-hydroxyphenylpropionylamide derivative (**8 in Figure 1A**) [10].

3. Lipid-modified CD1d ligands with amide groups

We recently focused on Ser28 in the hydrophobic pocket of CD1d and found that the introduction of an amide group into the acyl chain of α-GalCer, which can interact with this polar amino acid residue via hydrogen bonds, greatly enhanced its cytokine-inducing activity, as shown in **Figure 2A** [11]. In particular, the introduction of an amide group into an α-GalCer derivative with a shortened acyl chain length has been reported to produce a ligand with Th2 selectivity and high binding affinity to CD1d, which is a CD1d ligand with unique activity rarely reported so far (**9d in Figure 1B**).

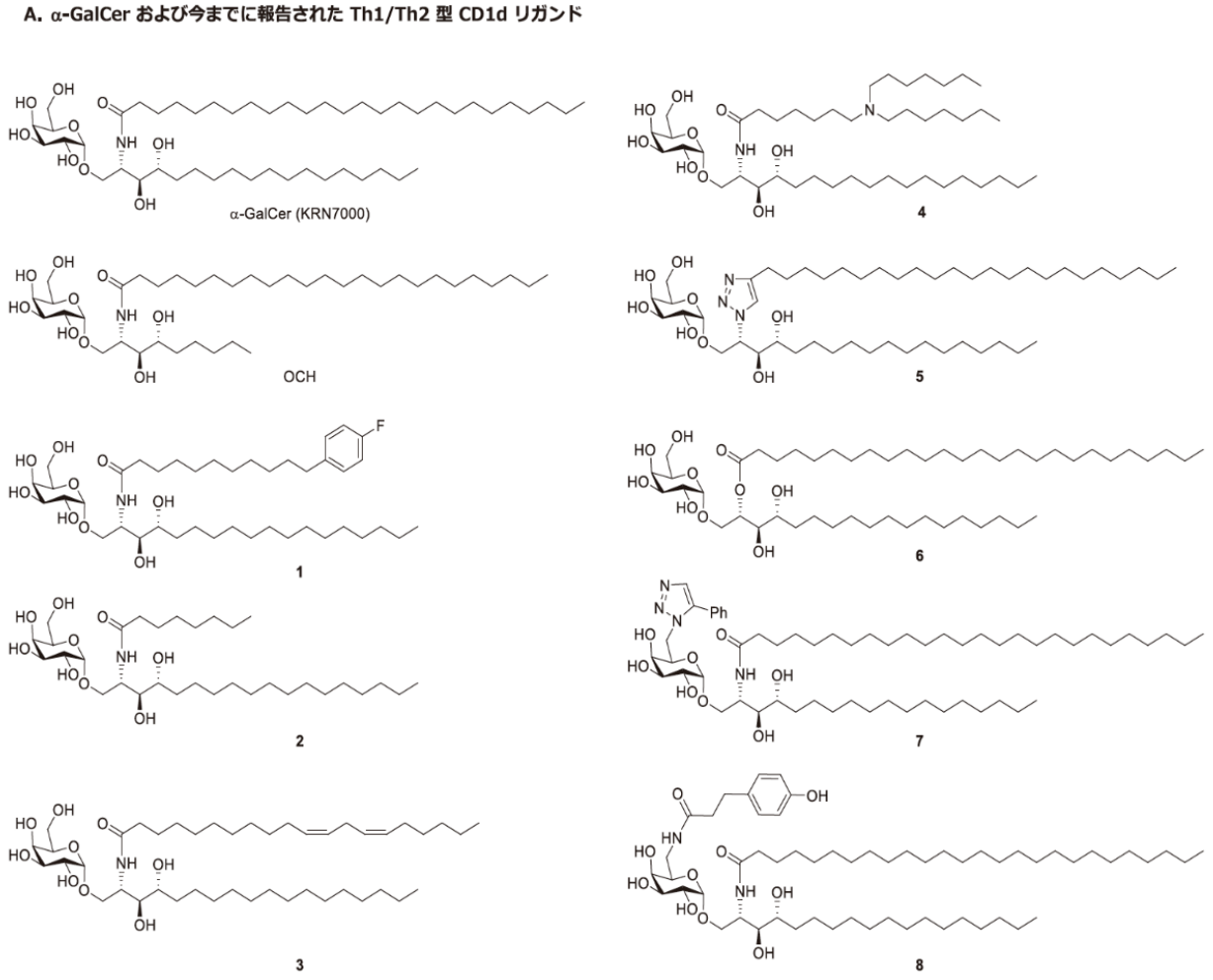


Figure 1. Structure of α-GalCer (KRN7000) and its derivatives

To evaluate the Th1/Th2 cytokine induction balance of the amide-containing ligands **9a-f**, we performed a cytokine induction assay using mouse splenocytes, quantified INF-γ and IL-4 and measured the ratio of Th1/Th2 cytokine induction. The ratio of these two cytokines was used as an index of Th1/Th2 selectivity, suggesting a tendency for Th2 selectivity to increase with decreasing acyl chain length (**Figure 2B**).

The binding affinity of each ligand to CD1d was evaluated using AlphaScreen™ (PerkinElmer Life Sciences). The results showed that **9b-f** in **Figure 1**, which has an amide group, bound to CD1d more strongly than α-GalCer, with **9b-d** in **Figure 1** having a higher affinity. Among these, **9b-d** in **Figure 1** was found to have a high binding affinity (**Figure 2C**) [12].

4. Mechanism of Th1/Th2 selectivity

As mentioned above, Th2-selective ligands have been developed by various groups, but the mechanism of their selectivity remains unclear. Wong *et al.* proposed that ligands with strong CD1d binding are Th1-selective [2]. On the other hand, Porcelli *et al.* proposed that CD1d ligands bind to CD1d by two pathways and that the difference between these pathways controls the balance of cytokines, and proposed that Th2-selective ligands bind directly to CD1d on the cell surface [13].

However, as mentioned in the previous section, our amide-containing ligand **9d** in **Figure 1** is a Th2-selective ligand with a high binding affinity to CD1d, and our results differ from those of Wong *et al.* There are few reports of Th2-selective and highly active CD1d ligands, and studies showing a relationship between Th1/Th2 selectivity and intracellular behavior are limited. To elucidate the mechanism of Th1/Th2 selectivity, we analyzed the intracellular behavior of ligand-CD1d complexes using amide-containing CD1d ligands that we have developed.

5. Analysis of the intracellular behavior of the ligand-CD1d complex [12]

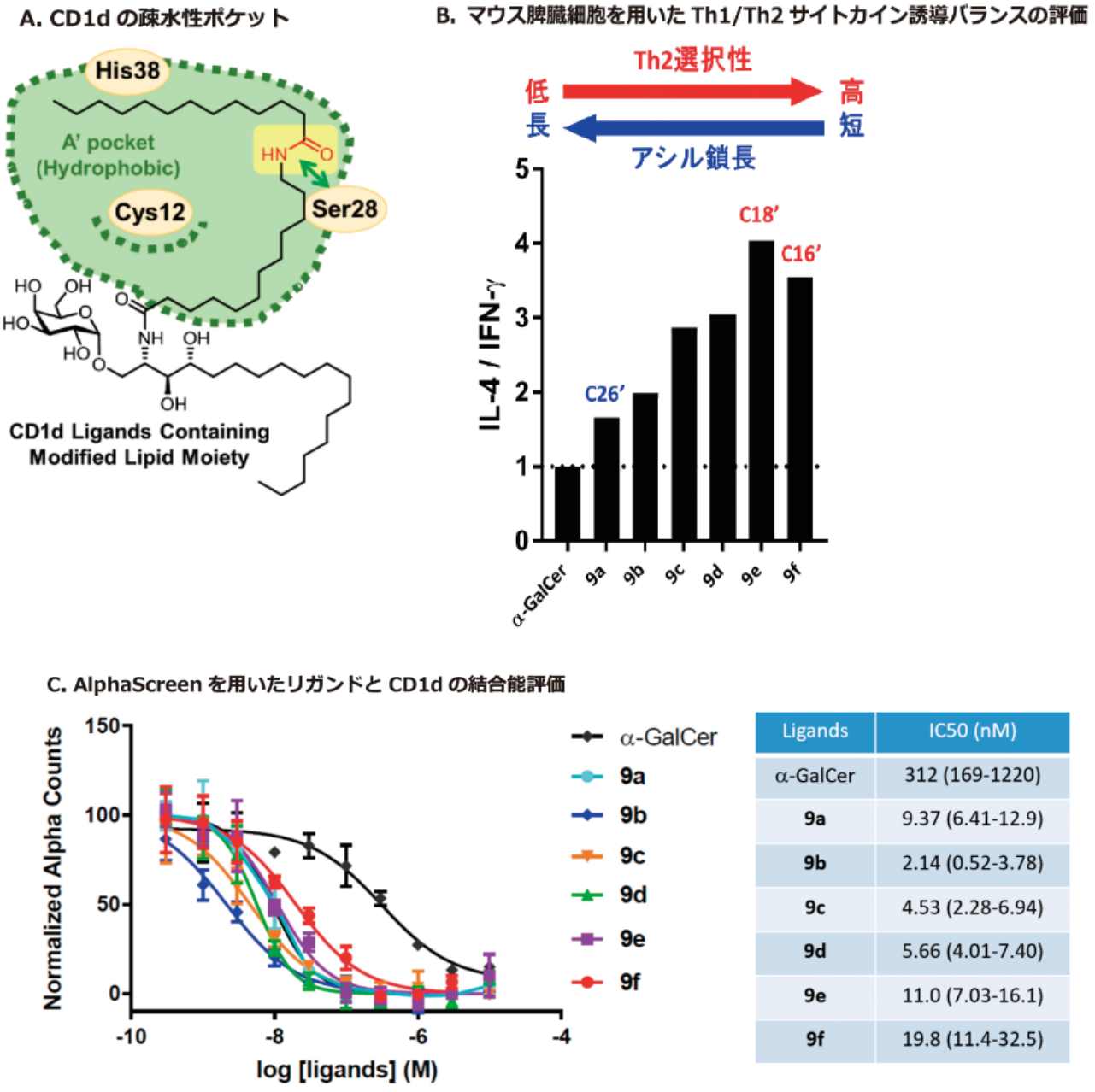
First, observations were made using confocal microscopy. RBL.CD1d cells with high CD1d expression were incubated with the ligand for 4 or 20 hours and then stained with an L363 antibody for observation, which recognizes the ligand-CD1d complex. In the case of α-GalCer and **9a**, which have long acyl chains, the ligand-CD1d complex (**Figure 3A**, orange) was observed on the cell surface only after 20 hours, whereas, in **9d** in **Figure 1**, which has short acyl chains, the complex was localized on the cell surface after 4 hours. The localization of the complex on the cell surface was observed at 4 hours.

A similar phenomenon was observed in flow cytometry analysis. The complexes of RBL.CD1d cells on the cell surface were quantified at each time point using the L363 antibody. As shown in **Figure 3B**, the results showed that the complexes on the cell surface gradually increased with time for α-GalCer and **9a** in **Figure 1**, which have longer acyl chain lengths, whereas for **9d** in **Figure 1**, which has shorter acyl chain lengths, the maximum value was reached at an earlier time.

These results suggest that the Th2-selective short-chain amide-containing ligand **9d** in **Figure 1** binds directly to CD1d on the cell surface. This result is consistent with that of Porcelli *et al.* and the Th1/Th2 selectivity was found to be influenced by the speed of presentation of the ligand on the cell surface.

6. Analysis of the intracellular behavior of the CD1d ligand

To further observe the behavior of the CD1d ligand itself, lipid-modified CD1d ligands with fluorescent groups were synthesized. These were added to RBL.CD1d cells and live cell imaging using confocal



Ligands	IC50 (nM)
α-GalCer	312 (169-1220)
9a	9.37 (6.41-12.9)
9b	2.14 (0.52-3.78)
9c	4.53 (2.28-6.94)
9d	5.66 (4.01-7.40)
9e	11.0 (7.03-16.1)
9f	19.8 (11.4-32.5)

Figure 2. The hydrophobic pocket of CD1d, evaluation of Th1/Th2 cytokine induction balance using mouse splenocytes, evaluation of ligand-CD1d binding ability using AlphaScreen. Figures partly modified and reprinted by permission from RightsLink: John Wiley and Sons, Angewandte Chemie International Edition, Copyright © 2018 [12].

microscopy revealed that the intracellular behavior of the ligands themselves differed depending on the acyl chain. In particular, the Th2-selective **9d** in **Figure 1** fluorescent labels localized faster on the plasma membrane, suggesting that the ligands that localize faster on the plasma membrane are Th2-selective.

7. Conclusion

We have developed Th2-selective and highly active CD1d ligands and found that the speed of ligand presentation on the cell surface influences Th1/Th2 selectivity. The development of these ligands is expected to advance our understanding of the

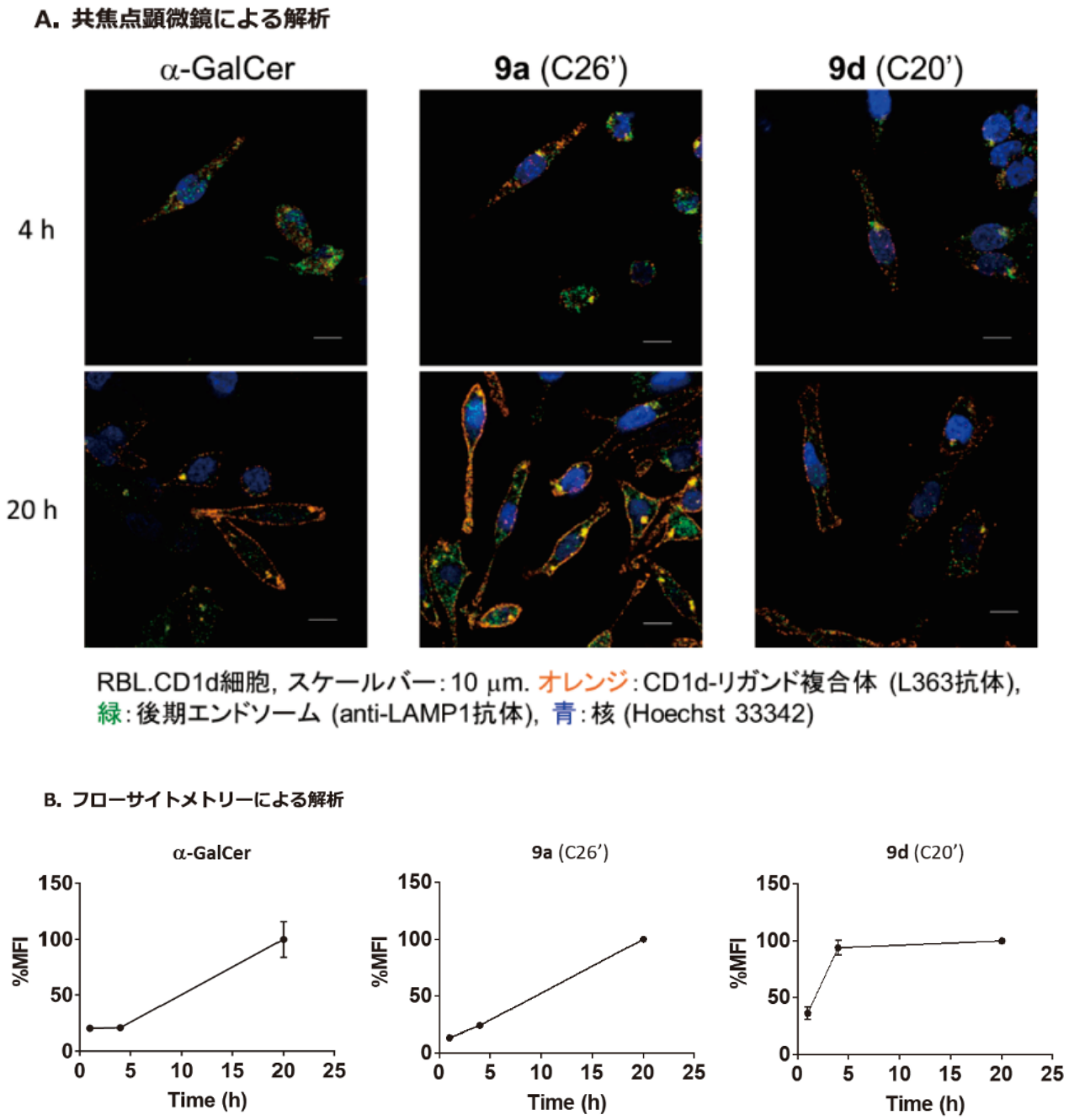


Figure 3. Analysis of the intracellular behavior of ligand-CD1d complexes. Figures partly modified and reproduced by permission from RightsLink: John Wiley and Sons, Angewandte Chemie International Edition, Copyright © 2018 [12].

mechanism of Th1/Th2 selectivity, and lead to the development of therapeutic agents and vaccine adjuvants for autoimmune and other diseases by controlling selectivity.

Disclosure statement

The authors declare no conflicts of interest.

References

[1] Morita M, Motoki K, Akimoto K, et al., 1995, Structure-Activity Relationship of Alpha-Galactosylceramides Against B16-Bearing Mice. J Med Chem, 38: 2176–2187.

[2] Liang PH, Imamura M, Li X, et al., 2008, Quantitative Microarray Analysis of Intact Glycolipid-CD1d Interaction and Correlation with Cell-Based Cytokine Production. J Am Chem Soc, 130: 12348–12354.

[3] Miyamoto K, Miyake S, Yamamura T, 2001, A Synthetic Glycolipid Prevents Autoimmune Encephalomyelitis by Inducing TH2 Bias of Natural Killer T Cells. Nature, 413: 531–534.

[4] Goff RD, Gao Y, Mattner J, et al., 2004, Effects of Lipid Chain Lengths in Alpha-Galactosylceramides on Cytokine Release by Natural Killer T Cells. J Am Chem Soc, 126: 13602–13603.

[5] Yu KO, Im JS, Molano A, et al., 2005, Modulation of CD1d-Restricted NKT Cell Responses by Using N-acyl Variants of Alpha-Galactosylceramides. Proc Natl Acad Sci USA, 102: 3383–3388.

[6] Baek DJ, Lee YS, Lim C, et al., 2010, Rational Design and Evaluation of a Branched-Chain-Containing Glycolipid Antigen that Binds to CD1d. Chem Asian J, 5: 1560–1564.

[7] Lee T, Cho M, Ko SY, et al., 2007, Synthesis and Evaluation of 1,2,3-Triazole Containing Analogues of the Immunostimulant Alpha-GalCer. J Med Chem, 50: 585–589.

[8] Shiozaki M, Tashiro T, Koshino H, et al., 2010, Synthesis and Biological Activity of Ester and Ether Analogues of Alpha-Galactosylceramide (KRN7000). Carbohydr Res, 345: 1663–1684.

[9] Jervis PJ, Graham LM, Foster EL, et al., 2012, New CD1d Agonists: Synthesis and Biological Activity of 6”-Triazole-Substituted α-Galactosylceramides. Bioorg Med Chem Lett, 22: 4348–4352.

[10] Hu L, Zhao C, Ma J, et al., 2019, Design, Synthesis, and Evaluation of α-Galactopyranosylceramide Mimics Promoting Th2 Cytokines Production. Bioorg Med Chem Lett, 29: 1357–1362.

[11] Inuki S, Aiba T, Hirata N, et al., 2016, Isolated Polar Amino Acid Residues Modulate Lipid Binding in the Large Hydrophobic Cavity of CD1d. ACS Chem Biol, 11: 3132–3139.

[12] Inuki S, Kashiwabara E, Hirata N, et al., 2018, Potent Th2 Cytokine Bias of Natural Killer T Cell by CD1d Glycolipid Ligands: Anchoring Effect of Polar Groups in the Lipid Component. Angew Chem Int Ed Engl, 57: 9655–9659.

[13] Im JS, Arora P, Bricard G, et al., 2009, Kinetics and Cellular Site of Glycolipid Loading Control the Outcome of Natural Killer T Cell Activation. Immunity, 30: 888–898.