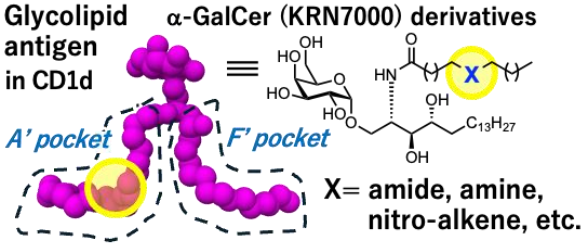
MONOGLYCOSYLCERAMIDE LIPID STRUCTURE-DEPENDENT CD1D FUNCTIONAL STABILIZATION AND SELECTIVE IMMUNOMODULATION

Kodai Sueyoshi, Kazunari Ueki, Nanako Kawate, Junichiro Kishi, Takanori Matsumaru, Yukari Fujimoto
Department of Chemistry, Graduate School of Science and Technology, Keio University, 3-14-1 Hiyoshi, Kohoku-ku Yokohama, Japan
fujimotoy@keio.jp

On antigen-presenting cells, the lipid antigen-presenting molecule CD1d recognizes glycolipid antigens and forms complexes that activate NKT cells, leading to the secretion of various cytokines that regulate immune responses. Recently, we have shown that endogenous monoglycosylceramides, such as GlcCer and GalCer, strongly bind to CD1d, stabilizing its structure while partially inhibiting its function. Among these, endogenous sphingosine-type β-GlcCer exhibited stronger binding affinity to CD1d than α-and β-GalCer [1]. Additionally, it was recently identified that dihydrosphingosine-based saturated α-GalCer serves as an endogenous active lipid antigen in mammals [2]. Meanwhile, through the exploration of lipid-modified KRN7000 type α-GalCer glycolipid antigens of CD1d, we also demonstrated that the binding affinity, cytokine induction activity and selectivity can be modulated by modifying the acyl group with polar functional groups [3], including covalent-ligand-type reactive group modification [3b].

To further investigate CD1d-ligand interactions, we developed highly Th2-biased glycolipids, performing synthesis and biofunctional evaluation of the novel α-GalCer derivatives [4], along with computational analysis to visualize the dynamics of the CD1d-ligand complex. Namely, for the synthesis of the novel α-GalCer derivatives with modified acyl groups with nitro group, we developed a novel synthetic method to introduce functional groups to the acyl groups, and achieved a highly efficient synthesis. The biological activities and functions of these derivatives were evaluated by binding affinity assays with the CD1d protein and cytokine induction assays using mouse splenocytes. Comparisons with previously reported amide- and amine-modified Th2-biased α-GalCer derivatives revealed that the newly synthesized compounds exhibit enhanced CD1d binding affinity and a significantly higher Th2-selective cytokine induction profile. Molecular dynamics (MD) simulations were also conducted to analyze the binding modes of these glycolipids, further elucidating their interactions with CD1d.



References:
1. K. Ueki, R. Nozawa, T. Matsumaru, S. Yamasaki, Y. Fujimoto, *ChemRxiv.* **2025**.
2. Y. Hosono, N. Tomiyasu, H. Kasai, S. Yamasaki, et al., *J. Exp. Med.* **2025**, 222, e20240728.
3. a) S. Inuki, E. Kashiwabara, N. Hirata, J. Kishi, E. Nabika, Y. Fujimoto, *Angew. Chem. Int. Ed.* **2018**, *57*, 9655-9659. b) J. Kishi, S. Inuki, E. Kashiwabara, N. Dohmae, Y. Fujimoto, et al., *ACS Chem. Biol.* **2020**, *15*, 353-359.
4. K. Sueyoshi, J. Kish, S. Inuki, T. Matsumaru, Y. Fujimoto, *Chem. Eur. J.* **2025**, *31*, e202403871.