

Structural variants of *Bacteroides fragilis*-derived alpha-galactosylceramides induce unique natural killer T cell responses

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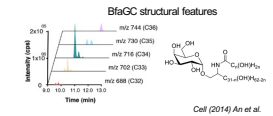
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ABSTRACT

Natural killer T (NKT) cells are a unique subset of thymus-derived T cells that recognize glycolipids presented by the CD1d molecule expressed on antigen-presenting cells. The most studied agonistic antigen of NKT cells is alpha-galactosylceramide (α GC). It has been shown that structural features of lipid antigens distinctly induce NKT cell responses, which emphasizes the importance of understanding the relationship between structural characteristics of lipid antigens and context-dependent NKT cell responses. In a previous study, we showed that human gut symbiont *Bacteroides fragilis* (*B. fragilis*) uniquely produces α GCs (BfaGC), which can normalize the overproliferation of neonatal colonic NKT cells in germ-free mice, and that chemically-synthesized structural isomers of BfaGCs induce structure-specific NKT cell responses. In this study, to further investigate the essential structural moiety of BfaGC for the unique immunomodulatory activity, we synthesized a panel of BfaGC structural variants by modifying their lipid chain structures and functional groups. We have examined how the variants differently impact NKT cell responses by analyzing effector cytokine production, NKT cell proliferation, relative binding affinities to CD1d, and preferential antigenic recognition by specific TCR $\text{V}\beta$ repertoires. We identified critical features and functional groups on the BfaGC structure leading to immunomodulatory outcomes. It is expected that our findings may advance further development of lipid antigens to fine-tune context-specific NKT cell responses in immunological diseases.

INTRODUCTION

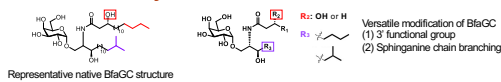
- NKT cell recognizes glycolipids presented by CD1d molecule expressed on antigen-presenting cells.
- The structural features of lipid antigens determine distinct NKT cell responses.
- The understanding of how NKT cell antigens regulate human diseases is still limited.
- Human gut resident bacteria *Bacteroides fragilis* (*B. fragilis*) uniquely produces structural variants of alpha-galactosylceramide (BfaGC).
- Structural isomers of BfaGCs induce structure-specific immuno-regulatory NKT cell responses.



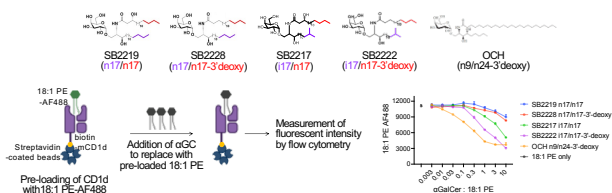
- Research Aim**
Study on the unique NKT cell responses induced by structural variants of BfaGCs

RESULTS

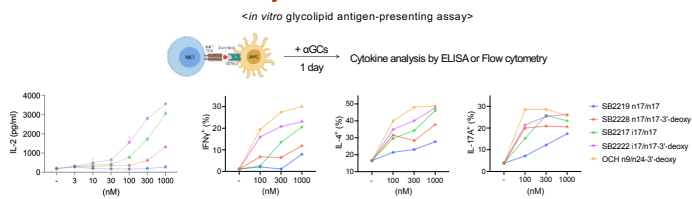
Chemical synthesis of BfaGC structural variants



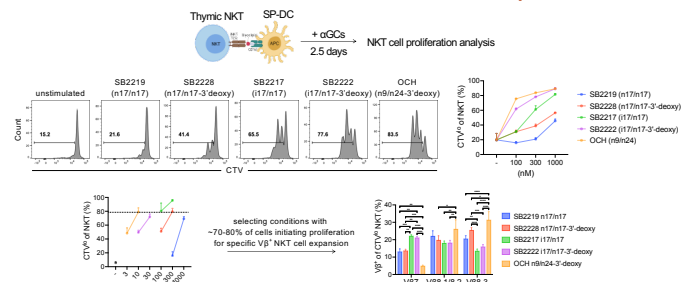
Structural variants of BfaGCs load onto CD1d with varying relative affinities.



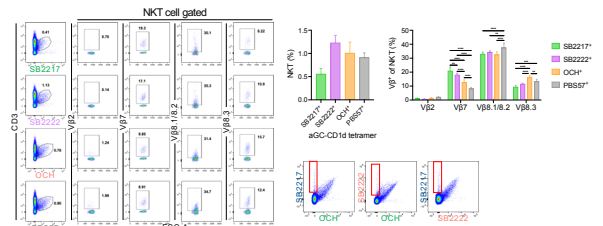
Differential NKT cell-mediated cytokine responses induced by structural variants of BfaGCs



BfaGC structural variants drive distinct in vitro NKT cell proliferation.



Unique NKT cell populations recognizing BfaGCs



SUMMARY

- BfaGC structural variants exhibited distinct relative binding affinities to CD1d, as measured by a competitive displacement assay, and differentially induced NKT cell cytokine responses.
- Sphinganine chain branching and the 3' hydroxyl group of BfaGCs are critical structural determinants of BfaGC-mediated NKT cell responses
- Unique NKT cell subpopulations defined by preferential TCR $\text{V}\beta$ repertoire usage were identified for each structural variant using α GC-CD1d tetramers

FURTHER STUDIES

- Phenotypic and functional characterization of BfaGC-recognizing NKT cells
- Characterization, via TCR sequencing, of specific TCR clonotypes within the $\text{V}\beta$ -preferential NKT subpopulations that selectively recognize BfaGC variants
- Assessment of NKT cell functional heterogeneity by correlating distinct TCR repertoires with the cytokine expression profiles of individual NKT cells.