Development of a Human, Cell-based Assay to study Lipids in Allergic Sensitisation

Georgie Hopkins

PhD Student at University of Nottingham

What is an Allergy?

- An allergy is an **unnecessary immune response** to a harmless substance e.g. peanuts.

- IgE-mediated allergies are **increasing** in prevalence, with IgE-mediated food allergies affecting up to 10% of children and 6% of adults worldwide [1-3] .

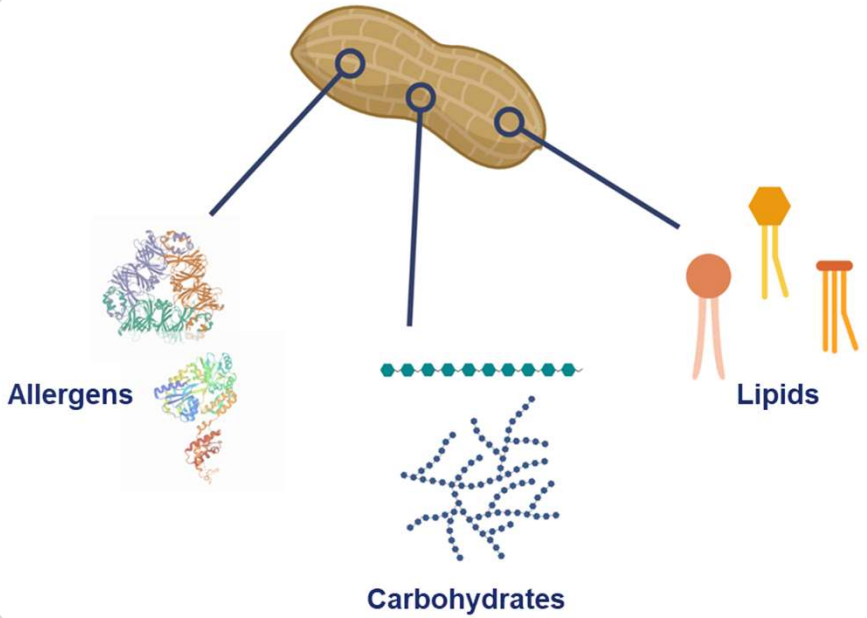
- Clinical manifestations:
  - Oedema
  - Hives
  - Itching
  - Vomiting
  - Anaphylaxis shock.

Copyright Allergyresearch. Reproduced under Creative Commons licence from Wikimedia.: https://commons.wikimedia.org/wiki/File:Hives_Urticaria.jpg

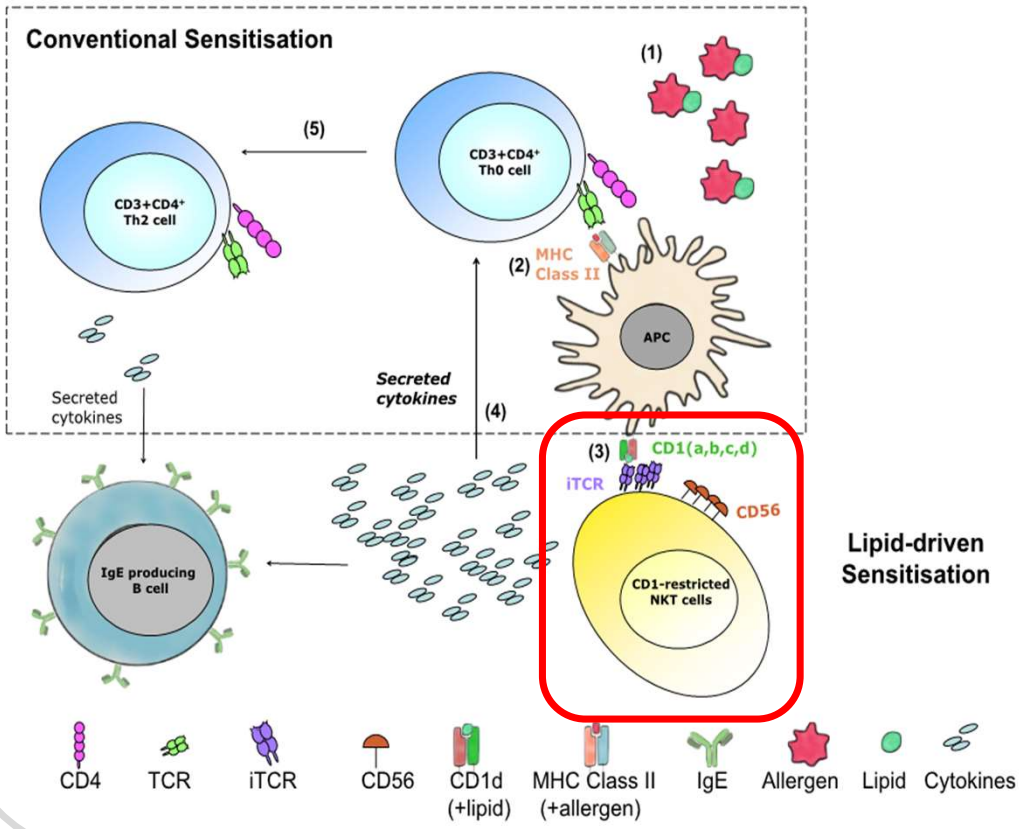
Copyright James Heilman. Reproduced under Creative Commons licence from Wikimedia: https://commons.wikimedia.org/wiki/File:Angioedema2010.JPG

1. Lee S. IgE-mediated food allergies in children: prevalence, triggers, and management. Korean J Pediatr. 2017;60(4):99-105.
2. Osborne NJ, Koplin JJ, Martin PE, Gurrin LC, Lowe AJ, Matheson MC, et al. Prevalence of challenge-proven IgE-mediated food allergy using population-based sampling and predetermined challenge criteria in infants. The Journal of allergy and clinical immunology. 2011;127(3):668-76.e1-2.
3. Prescott SL, Pawankar R, Allen KJ, Campbell DE, Sinn J, Fiocchi A, et al. A global survey of changing patterns of food allergy burden in children. The World Allergy Organization journal. 2013;6(1):21.

Lipids in Allergic Sensitisation



There is limited research on the role of lipids in allergic sensitisation, with only 19 papers published to date [1].



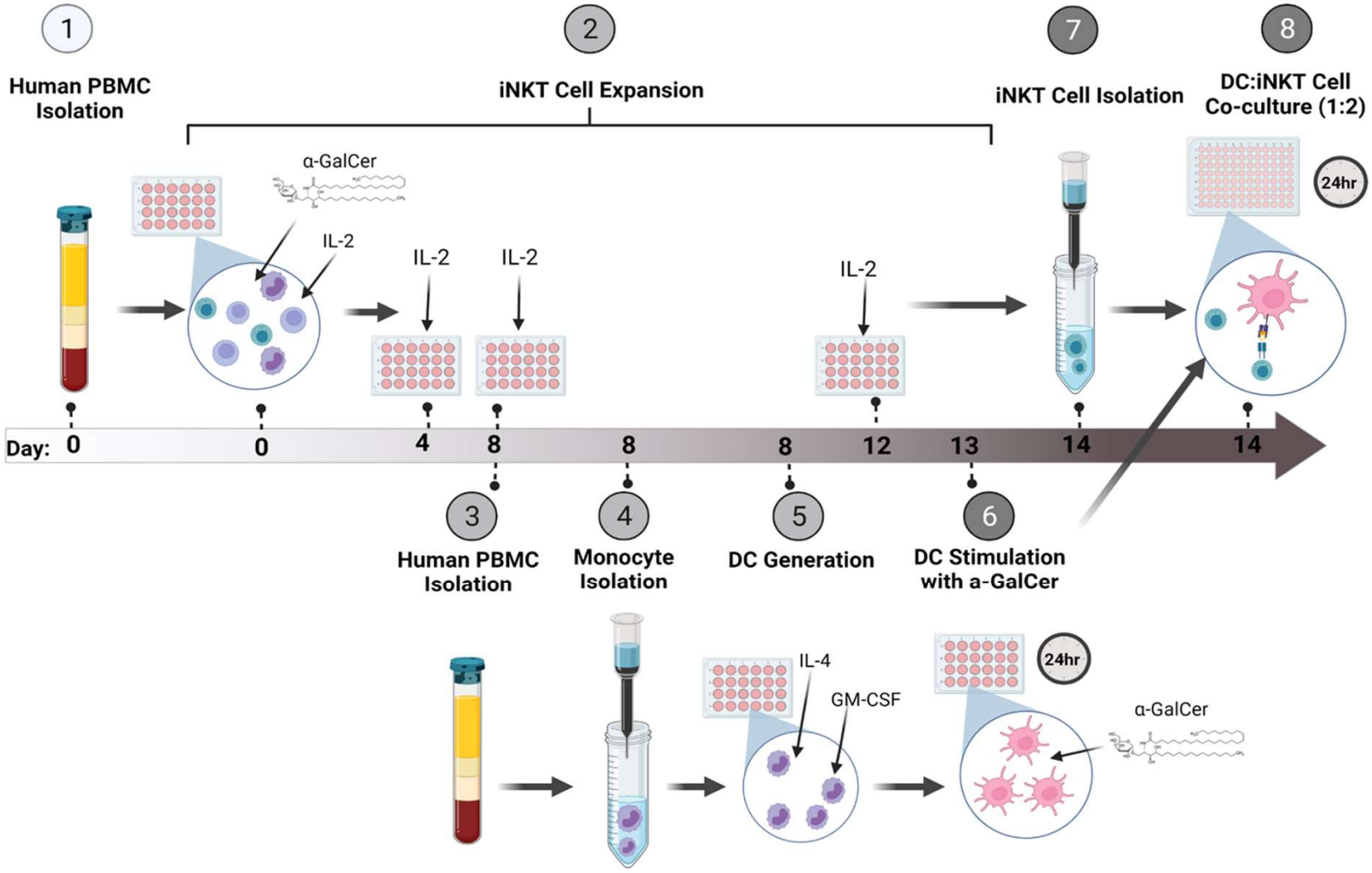
[1] Hopkins, G.V., et al., *The Role of Lipids in Allergic Sensitization: A Systematic Review.* Frontiers in Molecular Biosciences, 2022. **9**.

Aims

1. **To investigate the role of lipids in the development of allergic sensitisation, utilising a human model**
   - Measure Th1 and Th2 cytokine production from lipid-stimulated invariant NKT cells.
   - The lipid, α-GalCer, will be used in developing this assay as it as the most potent iNKT cell activator.

Methods

Assay Development



Obtaining blood from human participants was approved by The University of Nottingham's Medical School Ethics Committee (232-1902).

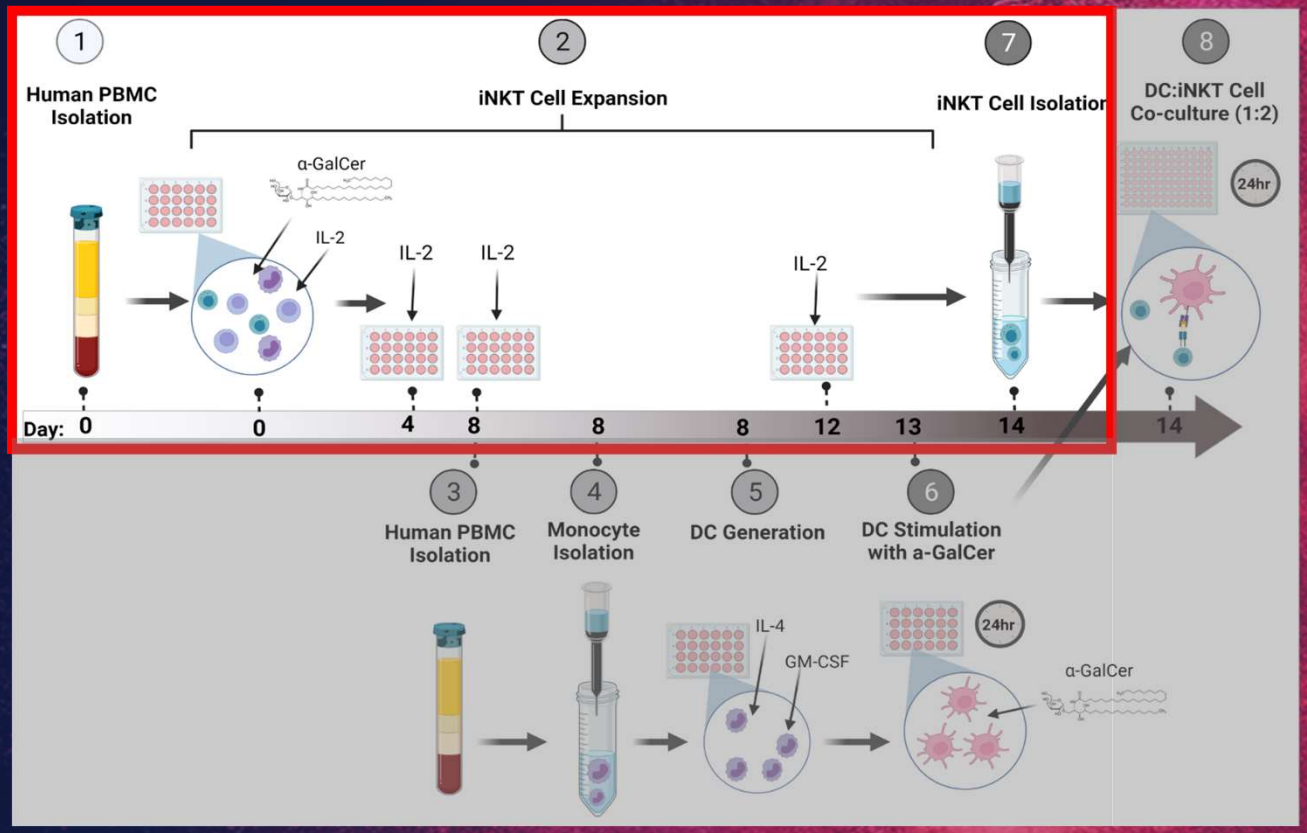
University of Nottingham
UK | CHINA | MALAYSIA

Unilever

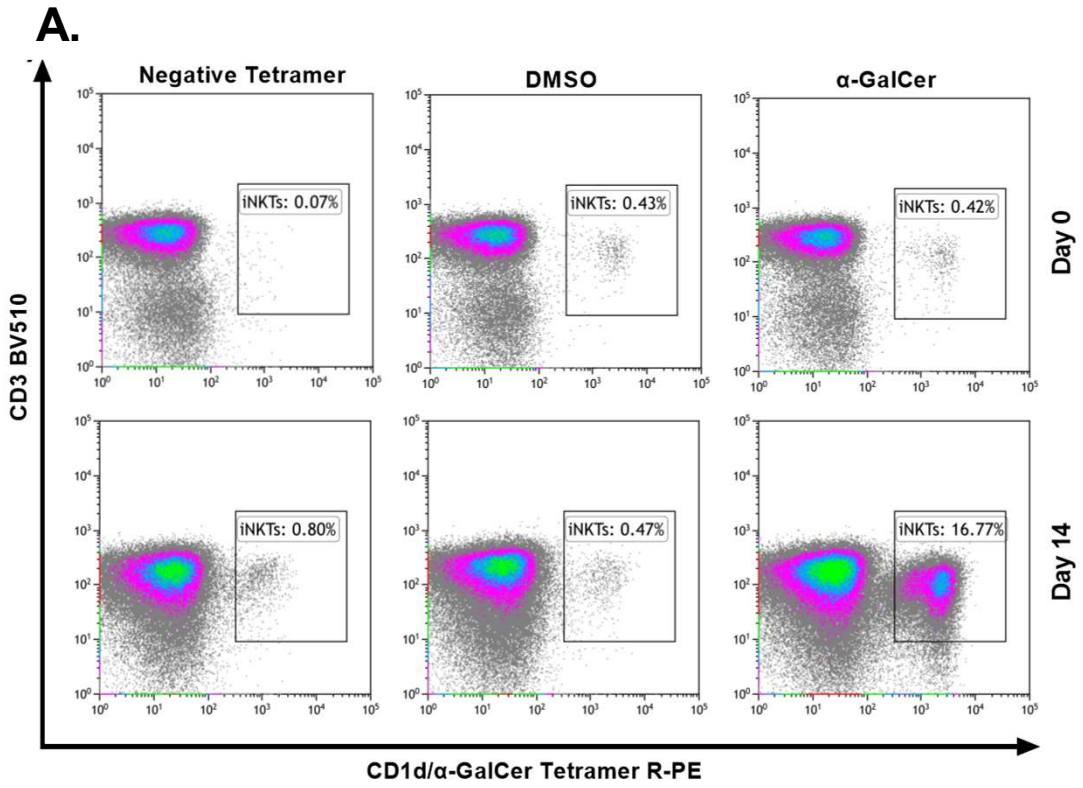
BBSRC
bioscience for the future

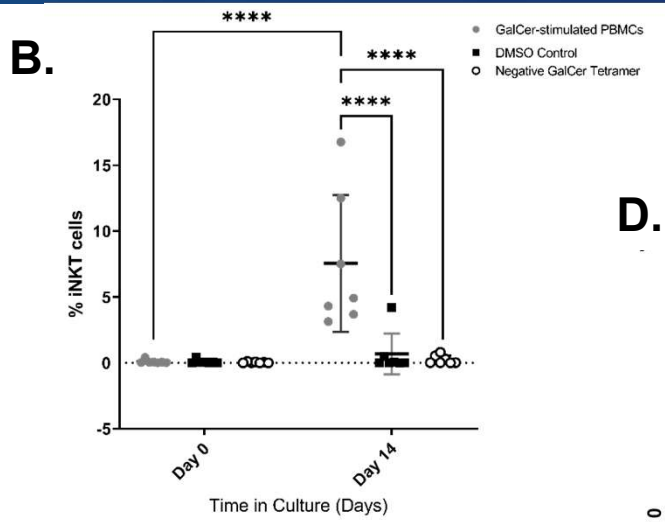
Results

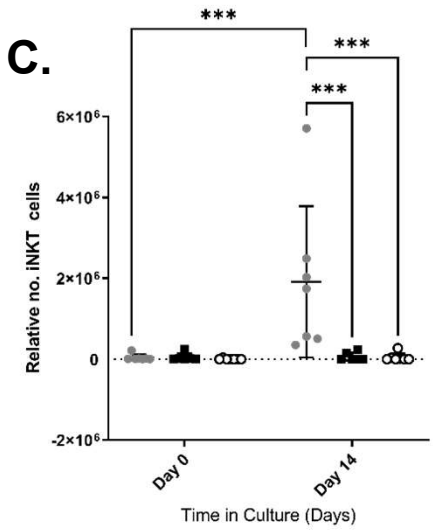
iNKT Cell Expansion and Isolation

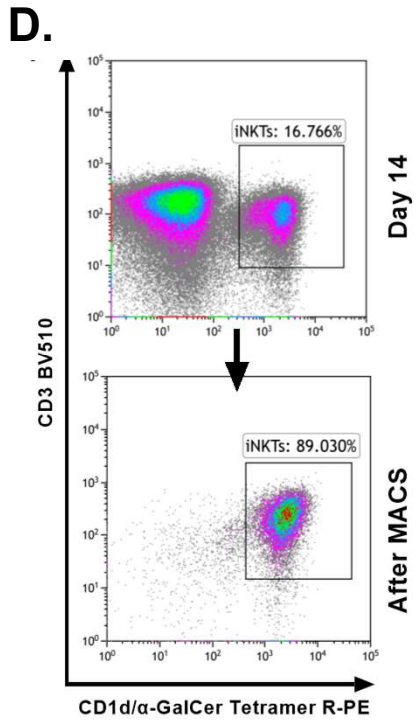


iNKT Cells Expanded by the Lipid, α-GalCer

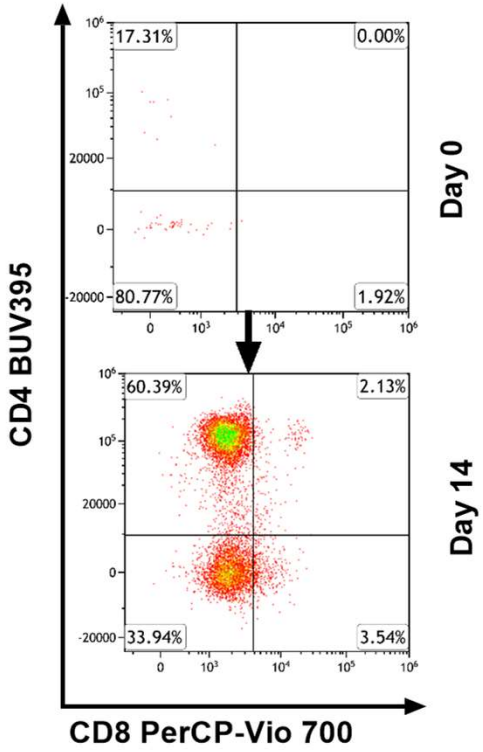


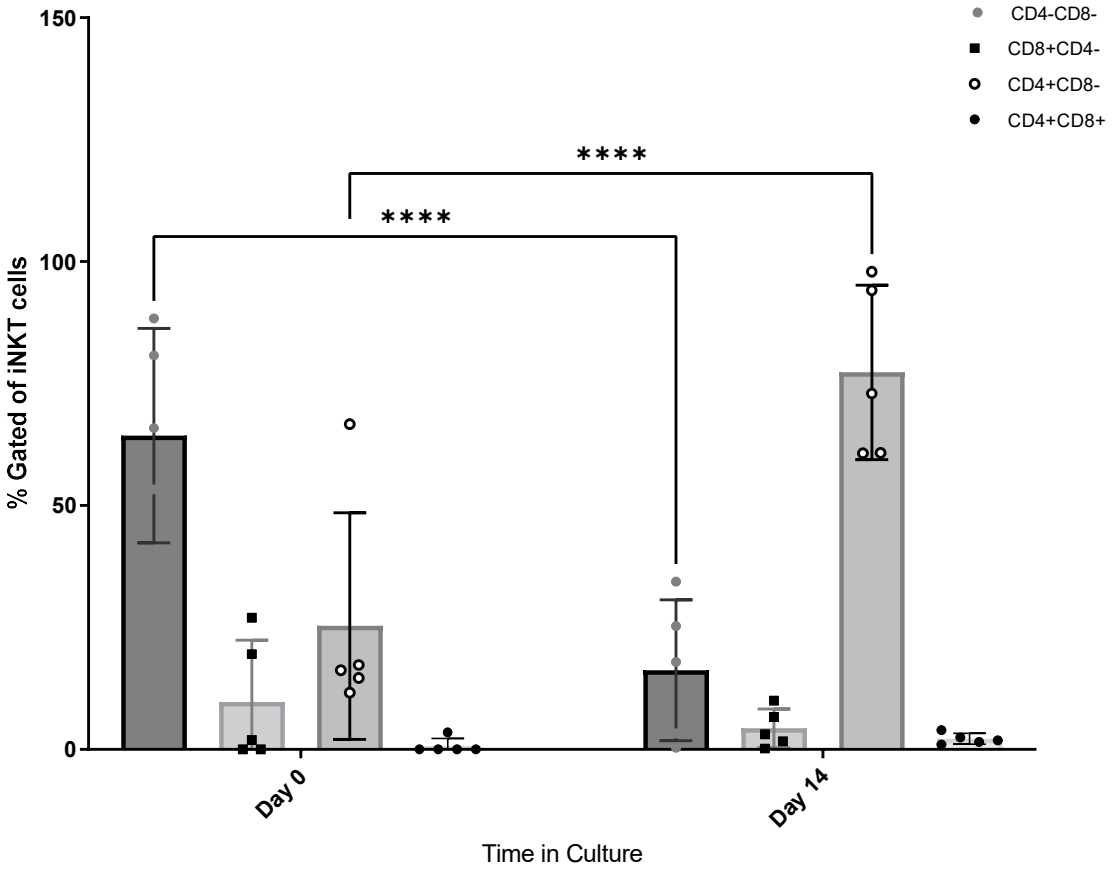






iNKT Cell Phenotype Shifts





The iNKT cell phenotype shifts from predominantly a **CD4-CD8-** phenotype at Day 0, to predominantly **CD4+CD8-** phenotype by Day 14 of expansion with α-GalCer and IL-2.

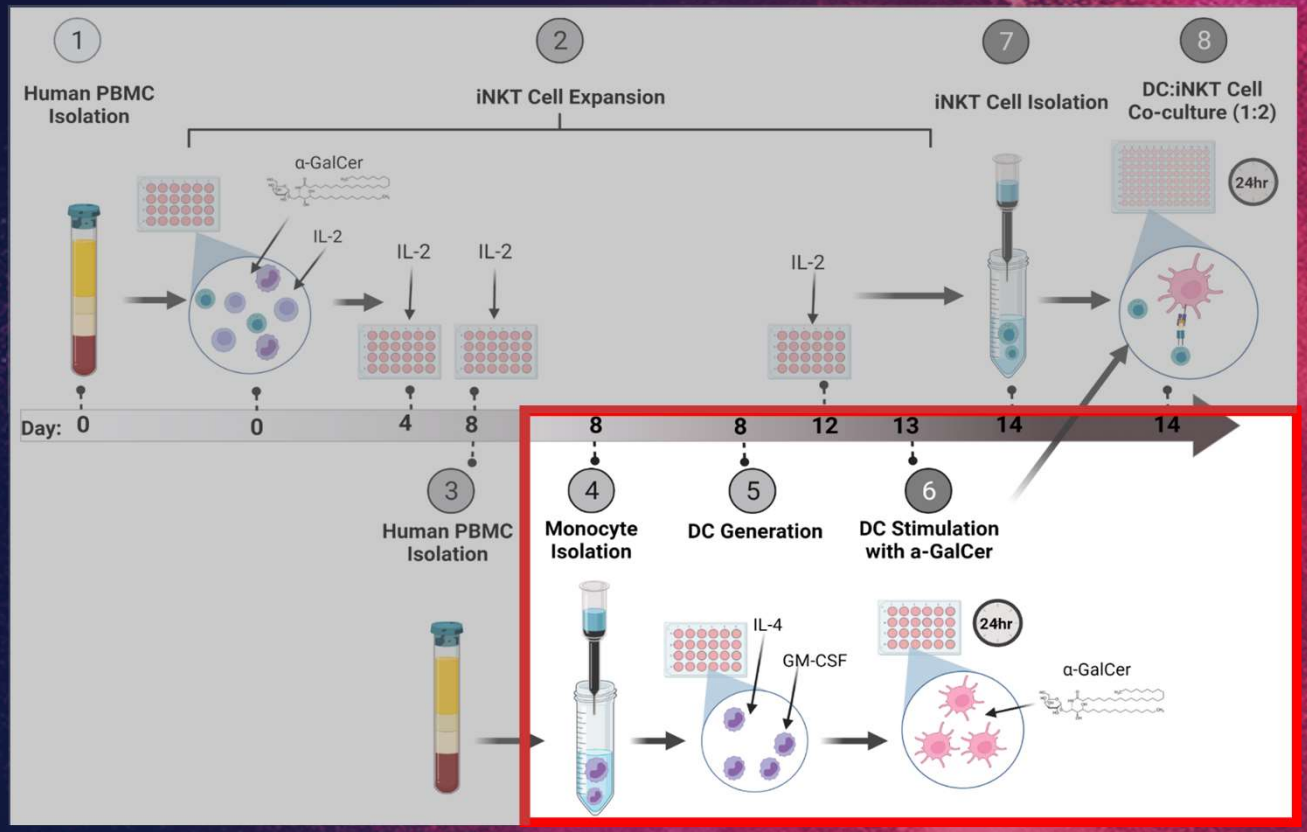
University of Nottingham
UK | CHINA | MALAYSIA

Unilever

BBSRC
bioscience for the future

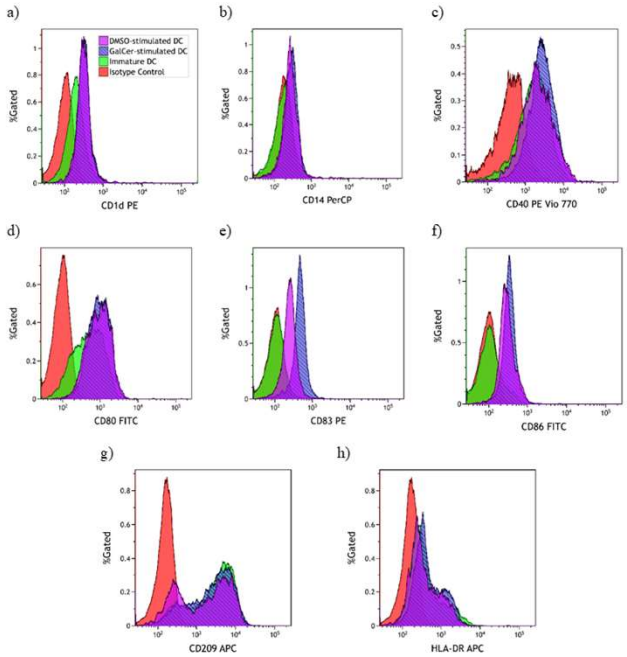
Results

DC Generation and Stimulation

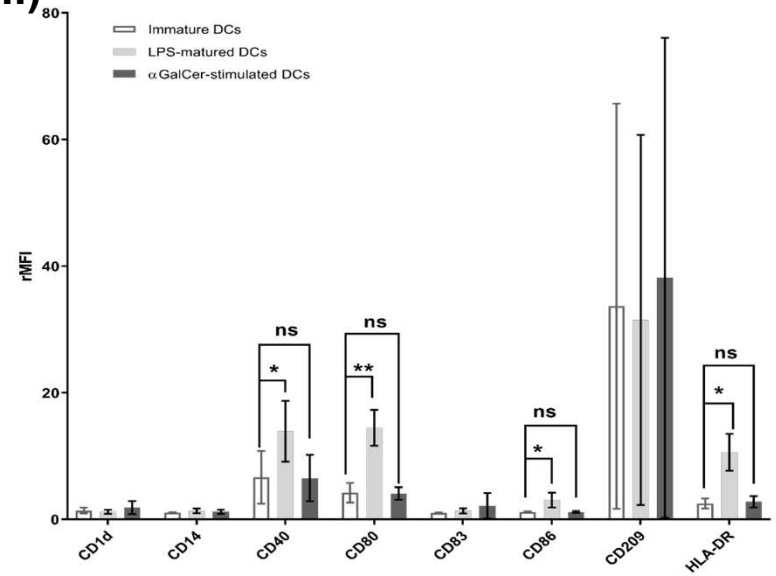


DCs Internalised the lipid, α-GalCer

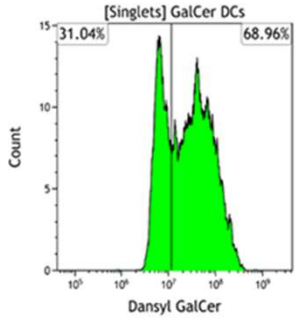
A. i)



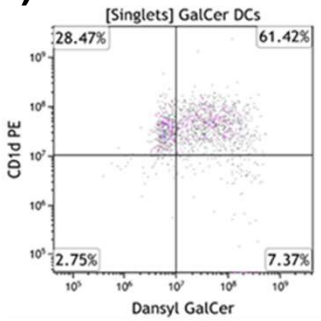
ii)



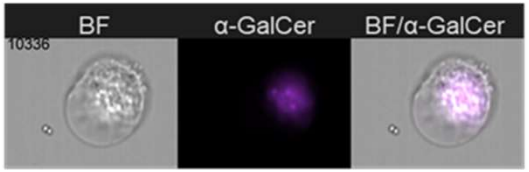
B. i)



ii)



C.



- Immature DCs were successfully generated and were matured using the standard method of LPS stimulation.
- The glycolipid, α-GalCer, did not mature DCs.
- CD1d expression not up regulated by α-GalCer.
- Fluorescent α-GalCer was internalised by immature DCs (iDCs).
- CD1d present on α-GalCer-pulsed DCs.

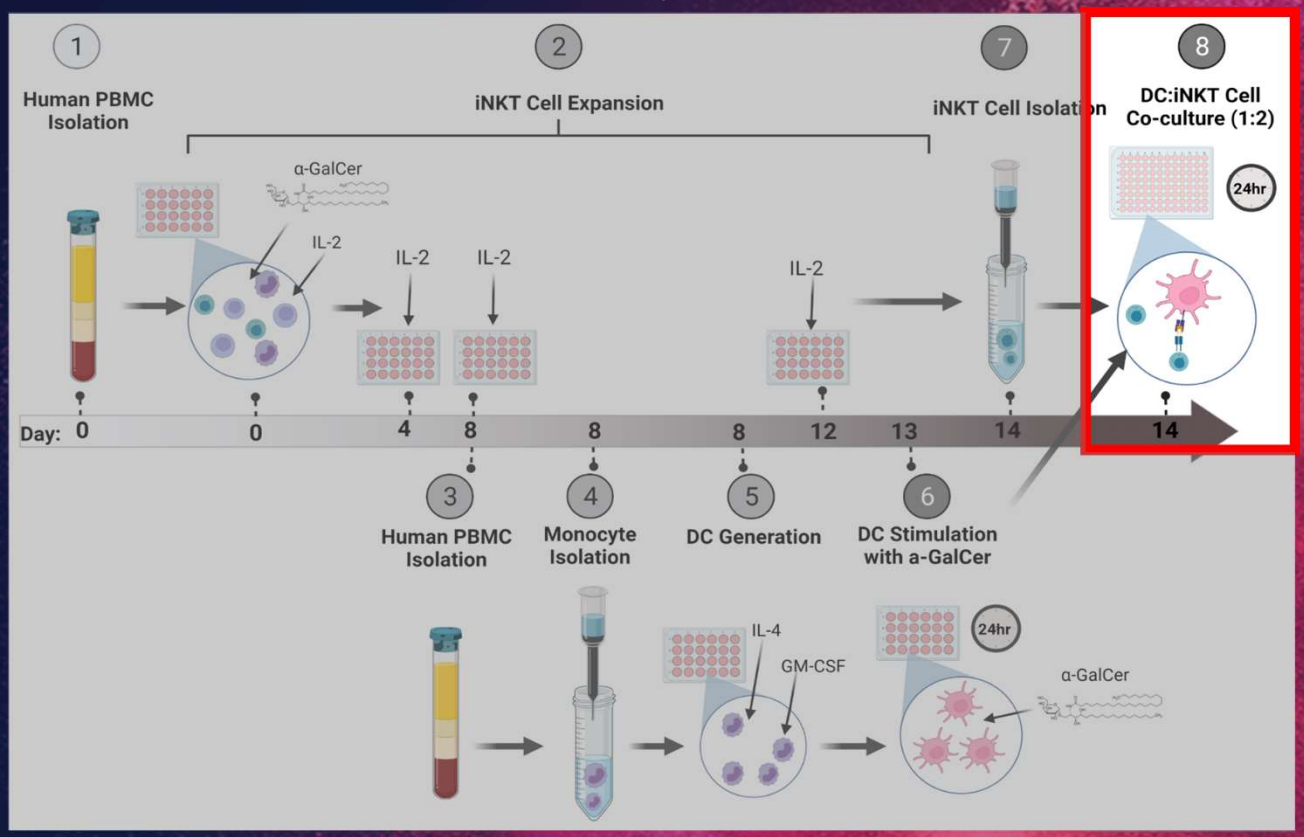
University of Nottingham
UK | CHINA | MALAYSIA

Unilever

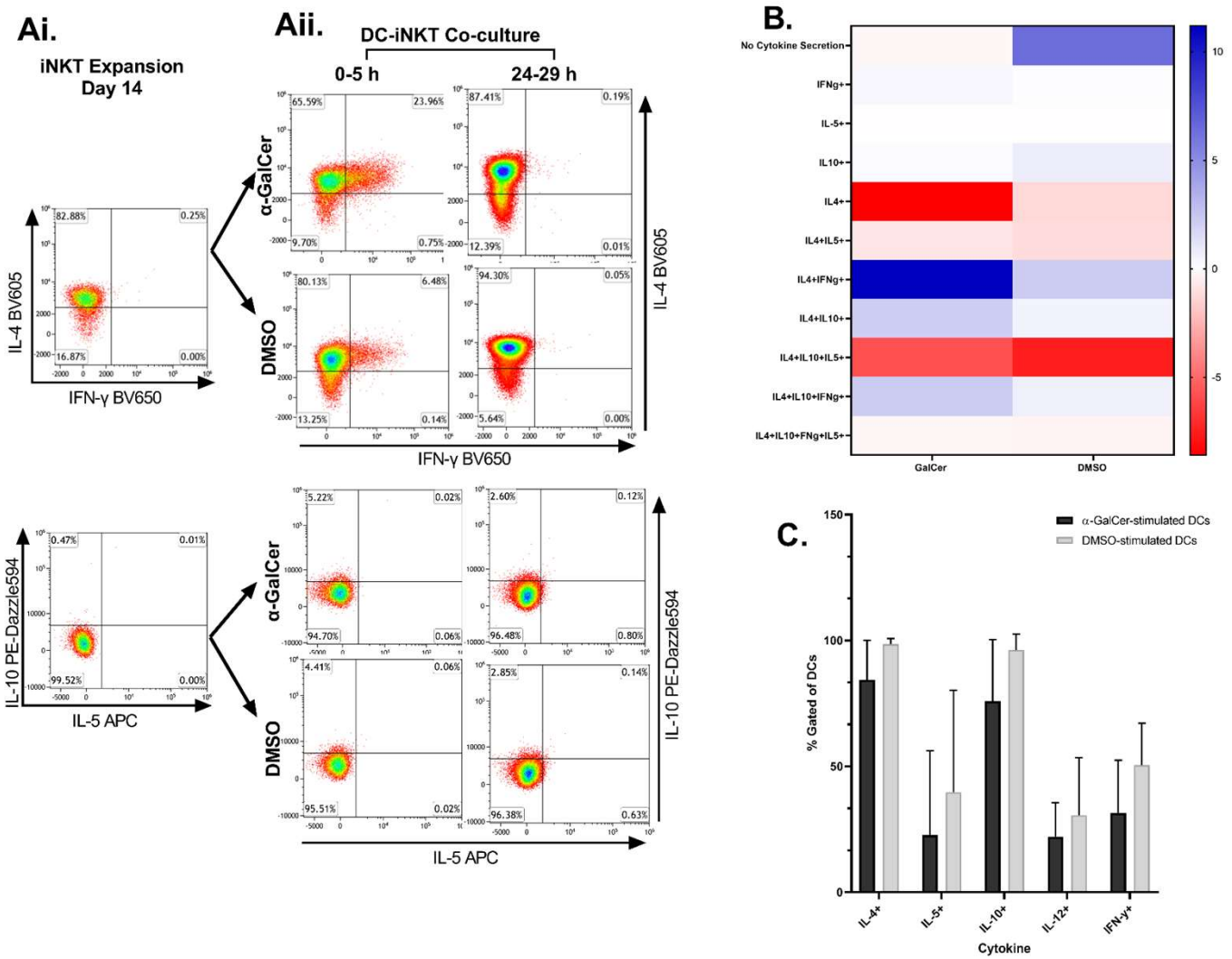
BBSRC
bioscience for the future

Results

Co-culture cytokine release



The lipid, α-GalCer, Increased IFN-γ and IL-4 Secretion




University of
Nottingham
UK | CHINA | MALAYSIA
Unilever
BBSRC
bioscience for the future
Conclusion

Conclusion

- Using the lipid a-GalCer, a model system was developed and optimised to measure iNKT cytokine responses.
- α-GalCer, increased Th1 and Th2 cytokine secretion of iNKT cells within 5 hours of stimulation.
- This system can be applied using lipids associated to food allergens, to investigate whether they also increase Th2 cytokine secretion, shifting to allergic sensitisation.
  - **Blood will be isolated from non-allergic and peanut allergic patients, and this co-culture experiment will be replicated, replacing the lipid a-GalCer with peanut lipids. Total and allergen-specific IgE will also be quantified by ELISA.**

Acknowledgements

Supervisors:
Prof Lucy Fairclough
Dr David Onion

Fairclough Lab:
Nancy Gomez
Davis Tucis
Laura Bartlett
William Browne
Silvia Granata

Tyler Harvey-Cowlishaw
Hannah Jackson
Graham Steers

Unilever:
Dr Stella Cochrane

Sutton Bonnington:
Dr David Gray
Dr Mohamed Gedi
Malgorzata Walczak

Sponsors:

University of Nottingham
UK | CHINA | MALAYSIA

Unilever

BBSRC
bioscience for the future

Thanks for listening!

Any Questions?

Georgie Hopkins