Lipidomic Changes Associated with Ether Lipid Deficiency in Germinal Centers of Spleen: A Multimodal IMS Approach

Marissa A. Jones [1,2], Sung Hoon Cho[3], Nathan Heath Patterson [1,4], Raf Van de Plas[5] Clay F. Semenkovich [6-8], Mark R. Boothby [3,9-12], Jeffrey M. Spraggins [1,2,4], Richard M. Caprioli [1,2,4,9,12]

[1]Mass Spectrometry Research Center, [2]Department of Chemistry, Vanderbilt University, Nashville, TN; [4]Department of Pathology, Microbiology and Immunology, School of Medicine, Vanderbilt University Medical Center, Nashville.; [4]Department of Biochemistry, Vanderbilt University, Nashville, Tennessee; [5]Department of Electrical Engineering, Katholieke Universiteit Leuven, Kasteelpark Arenberg 10, 3001 Leuven, Belgium; [6]Division of Endocrinology, Metabolism & Lipid Research, Washington University School of Medicine, Saint Louis, MO; [7]Division of Biology and Biomedical Sciences, Washington University School of Medicine, Saint Louis, MO; [8] Department of Cell Biology and Physiology, Washington University School of Medicine, Saint Louis MO, USA; [9]Department of Medicine, Vanderbilt University, Nashville, Tennessee; [10]Department of Cancer Biology, Vanderbilt University, Nashville, Tennessee ; [11] Vanderbilt-Ingram Cancer Center, Vanderbilt University, Nashville, Tennessee; [12] Department of Pharmacology, Vanderbilt University, Nashville, Tennessee

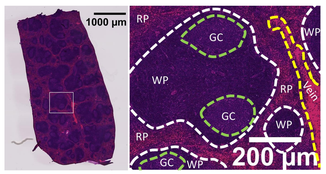
OVERVIEW

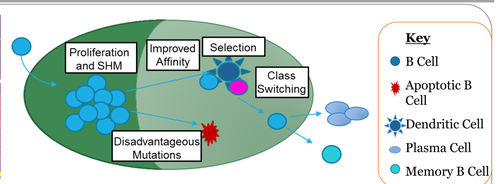
- Germinal centers (GCs) are sites of B cell proliferation, activation-induced cytidine deaminase (AID) hyper expression, selection, and differentiation formed during **humoral immune** responses. GCs are vital for generating high-affinity antibodies (Ab) and durable Ab secretion.
- Multimodal imaging incorporating Matrix-assisted laser desorption/ionization (MALDI) imaging mass spectrometry (IMS), autofluorescence/fluorescent emission (Fem), immunofluorescence (IF), and hematoxylin and eosin (H&E) staining, enables the correlation of biological structures to lipid localization.
- Many ether lipids were found to localize to GCs. This finding led us to **hypothesize** that GCs have **enhanced peroxisomal activity** and this organelle leads to the synthesis of ether lipids that may play a role in **hypoxic mediation**.
- PexRap, a peroxisomal enzyme that executes a late step in ether lipid synthesis, was eliminated in mice causing a **decrease in ether lipids;** the lipid localizations were investigated.

BACKGROUND

MALDI IMS is a technology that enables the unlabeled mapping of molecules directly from tissue sections. Ideal for untargeted discovery and spatial comparisons to histological data, the tool is adept for the study of the global lipidome. Lipidomics, a subset of metabolomics, allows for the investigation of the molecular products of metabolism, thus enhancing molecular insights into patient phenotypes. Lipid studies are specifically well suited for IMS due to lipid bioavailability, lipid bio-integration, and lipid involvement in structural components such as the cell membranes which define each cell.

BIOLOGICAL RELEVANCE

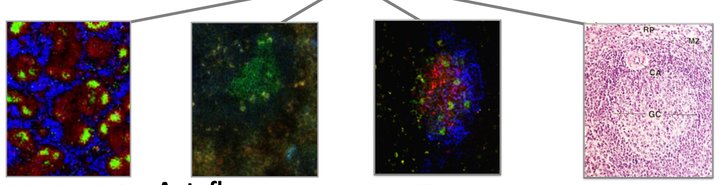




Adaptive immunity, the lymphocyte-dependent component of mammalian responses to pathogens, is essential for vaccine efficacy and mediates a variety of diseases. GCs are the histological features in which antibody genes are mutated to improve selectivity to a pathogen. The GC light zone is the site of antibody selection and determines the fate of the B cell. The GC dark zone is the site of proliferation and mutation of B cells. It was recently discovered that the GC light zone is **hypoxic**.[1]

MULTIMODAL IMAGING RELEVANCE

Biological Imaging Modalities



MALDI IMS
- High molecular specificity
- No tags
- Multiplexed
- Lower spatial res.

Autofluorescence
- Non-destructive
- Enables high accuracy alignment
- High spatial resolution

IF
- High chemical specificity and spatial resolution
- Non-MALDI IMS compatible
- Light and dark zone identification

H&E Staining
- Low chemical specificity[2]
- Gold standard
- High spatial res.
- GC identification

ACKNOWLEDGEMENTS

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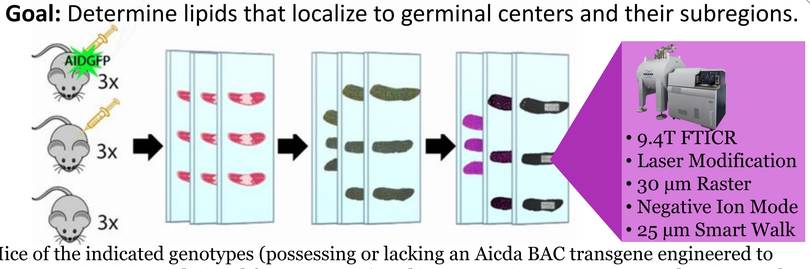
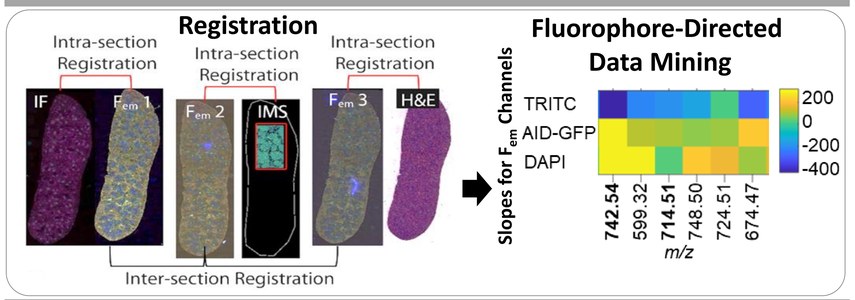
DISCOVERY EXPERIMENTAL DESIGN

Goal: Determine lipids that localize to germinal centers and their subregions.

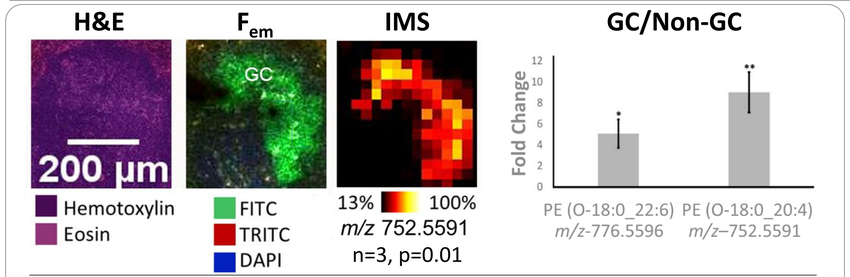


Mice of the indicated genotypes (possessing or lacking an Aicda BAC transgene engineered to express AID-GFP translational fusion protein) and immunization status were used at 6–7 weeks of age. Spleens harvested 8 d postimmunization were used to generate triads of serial tissue sections (12 µm thickness) followed by fluorescence emission (F_{em}) and other imaging modalities.

DATA ANALYSIS



RESULTS

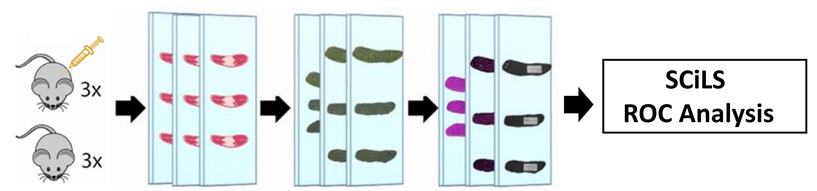


m/z	Lipid ID	DB Matches	P value GC vs. non-GC	P value LZ vs. DZ	ppm error*	Image Fusion Slope
671.4647	PA(18:1_16:1)	6	0.09	0.0007	0.0700	222.1
699.4957	PA(18:1_18:1)	6	0.03	0.0002	0.3617	277.2
699.4957	PA(18:0_18:2)	7	0.03	0.0002	0.3617	277.2
699.4957	PA(20:2_16:0)	7	0.03	0.0002	0.3617	277.2
714.5069	PE(18:2 16:0)	4	0.04	0.2	0.0532	102.5
716.5224	PE(18:0_16:1)	3	0.1	0.9	0.0586	243.8
725.5120	PA(20:3_18:0)	8	0.007	0.02	0.5334	63.2
740.5246	PE(18:1_18:2)	4	0.01	0.01	2.8872	112.8
742.5389	PE(18:0_18:2)	5	0.04	0.0006	1.0478	290.5
746.5130	PE(P-16:0_22:6)	6	0.005	<0.0001	1.4641	280.0
748.5273	PE(O-16:0_22:6)	6	0.007	0.2	0.3701	236.6
752.5591	PE(O-18:0_20:4)	5	0.01	<0.0001	0.3229	219.0
762.5088	PE(16:0_22:6)	4	0.03	0.2	2.5810	167.0
772.5314	PE(P-18:1_22:6)	5	0.03	0.01	5.0005	163.3
776.5596	PE(O-18:0_22:6)	5	0.05	<0.0001	0.8795	244.7
786.5303	PS(18:0_18:2)	8	0.02	0.0004	2.9771	279.4
812.5460	PS(18:0_20:3)	2	0.03	0.3	2.9771	37.1
857.5182	PI(16:0_20:4)	16	0.009	0.002	0.8210	400.6
883.5360	PI(18:1_20:4)	6	0.003	0.1	3.2528	565.8
887.5609	PI(18:0_20:3)	14	0.0006	0.07	3.9163	252.9

PA: Glycerophosphate, PE: Phosphatidylethanolamine, PS: Phosphatidylserine, PI: Phosphoinositol

HYPOTHESIS TESTING

Goal: Investigate the effect of an ether synthesis defect on germinal center lipids.

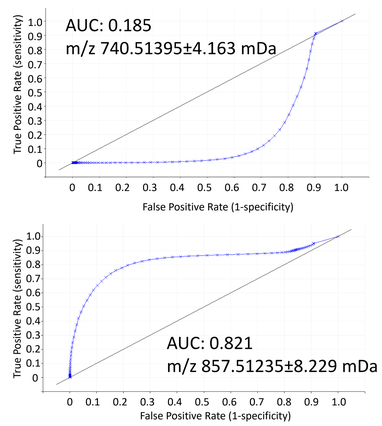


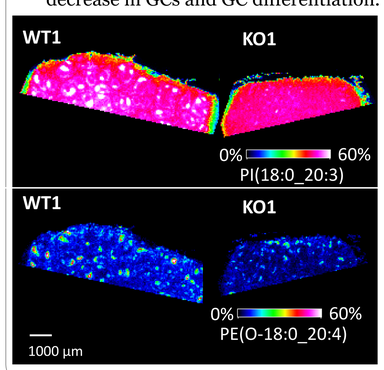
PexRap, a peroxisomal enzyme that executes a late step in ether lipid synthesis, was eliminated by using a tamoxifen-induced genetic system. Ten days after inducing generalized *Dhrs7b* deletion, adult C57/BL6 mice, wild-type (WT, n=4) and PexRAP-deficient (KO, n=3) were immunized with sheep red blood cells and sacrificed seven days later. Multimodal imaging (MALDI IMS, H&E, Fem, and IF) was performed. Data was further analyzed using a ROC analysis in SCiLS.

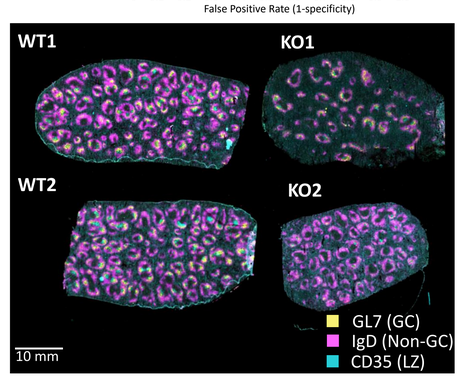
RESULTS

Ether and non-ether lipids are separated by receiver operator curve (ROC) analysis. IMS data was imported into SCiLS (Bruker Corp.) and root mean square normalized. Then imaging regions were grouped as WT or KO and compared using a ROC analysis.

- WT Spleen and KO spleen were separated based on ROC curve for ether and plasmalogen lipids.
- Ether lipids showed an ROC > 0.7 and non-ether lipids showed an ROC <0.4 corresponding to differing localization shifts.
- Ether lipids tended to show a change in size and number while non-ether GC lipids showed localization changes.
- In IF, KO mice showed a qualitative decrease in GCs and GC differentiation.







CONCLUSIONS

- Discovered **16 GC-specific** lipids including many **ether lipids.**
- Demonstrated the utility of **cell-type-specific** fluorescence for multimodal imaging and fluorophore-directed data mining.
- Generated the **hypothesis** that GCs have **enhanced peroxisomal activity** which leads to the synthesis of ether lipids that may play a role in **hypoxic mediation.**
- Showed that an **ether lipid synthesis defect affected GC lipid localization** that could be **elucidated** through ROC analysis.
- Correlated this ether lipid synthesis defect to qualitative **decreases in numbers of GCs and their sizes.**