Interrogation of the *i*NKT-MAIT cell interface

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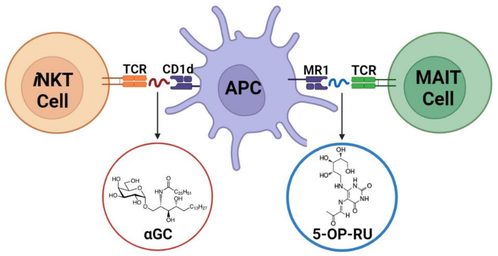
BACKGROUND

- Invariant natural killer T (*i*NKT) cells and mucosa-associated invariant T (MAIT) cells are unique subsets of T cells that express a highly conserved T cell receptor.
- Compared to conventional T cells, *i*NKT and MAIT cells are restricted to monomorphic major histocompatibility complex (MHC) class I-like molecules presenting non-peptidic antigens.
- Specifically, *i*NKT cells react to glycolipid antigens such as α-galactosylceramide (αGC) presented by CD1d, and MAIT cells recognize microbial vitamin B metabolites such as 5-(2-oxopropylideneamino)-6-D-ribitylaminouracil (5-OP-RU) presented by MHC-related protein 1 (MR1).

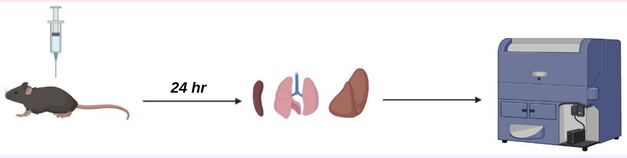


- Upon activation, both *i*NKT and MAIT cells regulate the function of many immune cell types downstream, including natural killer cells, B cells, regulatory T cells, and myeloid-derived suppressor cells.
- Despite their shared immunomodulatory properties, whether and how *i*NKT and MAIT cells regulate each other *in vivo* remains unknown.
- Identifying whether *i*NKT and MAIT cells regulate each other's function is an important question given that both cell subsets have a role in regulating tumor responses and display potent antimicrobial properties.

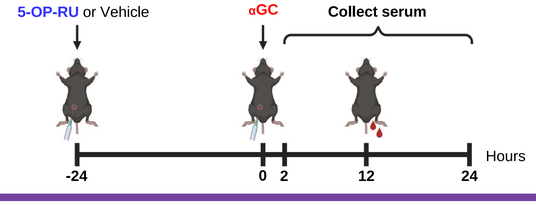
METHODS

*i*NKT and MAIT Cell Activation *in vivo*

Effect of *i*NKT cell activation on MAIT cell phenotype:	Inject B6-MAIT^{CAST} mice i.p. with **αGC** or vehicle	Isolate splenocytes and pulmonary and hepatic non-paranchymal mononuclear cells	Analyze **MAIT cell activation markers** (CD25, CD44, CD69) by flow cytometry
Effect of MAIT cell activation on *i*NKT cell phenotype:	Inject B6-MAIT^{CAST} mice i.p. with **5-OP-RU** or vehicle	Isolate splenocytes and pulmonary and hepatic non-paranchymal mononuclear cells	Analyze **iNKT cell activation markers** (CD25, CD44, CD69) by flow cytometry



Serum collection following MAIT cell-primed *i*NKT cell activation



RESULTS

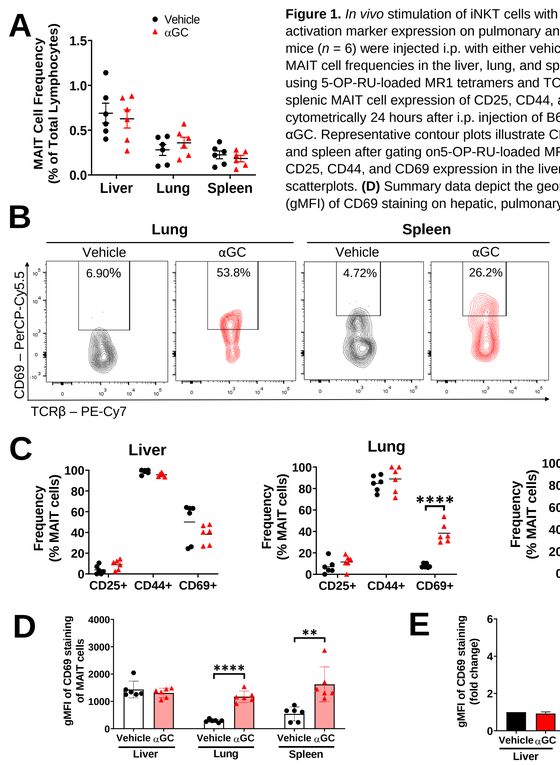


Figure 1. *In vivo* stimulation of iNKT cells with αGC leads to increased CD69 activation marker expression on pulmonary and splenic MAIT cells. **(A)** B6-MAIT^{CAST} mice (*n* = 6) were injected i.p. with either vehicle or αGC. Twenty-four hours later, MAIT cell frequencies in the liver, lung, and spleen were assessed by flow cytometry using 5-OP-RU-loaded MR1 tetramers and TCRβ mAbs. **(B)** Hepatic, pulmonary, and splenic MAIT cell expression of CD25, CD44, and CD69 was assessed flow cytometrically 24 hours after i.p. injection of B6-MAIT^{CAST} mice (*n* = 6) with vehicle or αGC. Representative contour plots illustrate CD69+ MAIT cell frequencies in the lung and spleen after gating on5-OP-RU-loaded MR1 tetramer+ events. **(C)** MAIT cell CD25, CD44, and CD69 expression in the liver, lung, and spleen are summarized as scatterplots. **(D)** Summary data depict the geometric mean fluorescence intensity (gMFI) of CD69 staining on hepatic, pulmonary, and splenic MAIT cells (*n* = 6). **(E)** gMFI of CD69 staining on hepatic, pulmonary, and splenic MAIT cells are depicted as an average fold change of three independent experiments (*n* = 2 per experiment). Each symbol in (A), (C), and (D) represents an individual mouse. Error bars represent SEM. Statistical analyses were performed using unpaired Student's t tests. *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001.

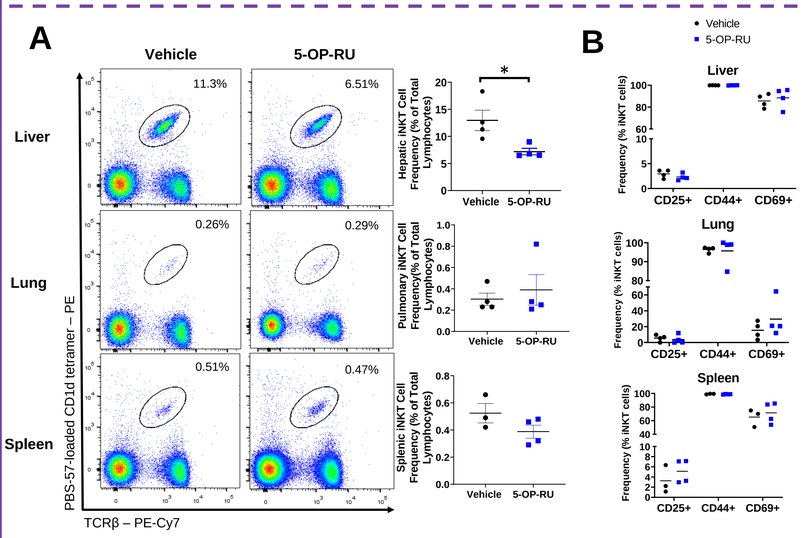


Figure 2. *In vivo* activation of MAIT cells with 5-OP-RU does not result in activation marker expression of hepatic, pulmonary, and splenic iNKT cells. **(A)** B6-MAIT^{CAST} mice (*n* = 4) were injected i.p. with either vehicle or 5-OP-RU. Twenty-four hours later, *i*NKT cell frequencies in the liver, lung, and spleen were assessed flow cytometrically using PBS-57-loaded CD1d tetramers and TCRβ mAbs. Representative dot plots are shown. **(B)** Flow cytometry was used to determine the expression of CD25, CD44, and CD69 on iNKT cells in the liver, lung, and spleen twenty-four hours following an i.p. injection of B6-MAIT^{CAST} mice (*n* = 4) with either vehicle or 5-OP-RU. Each symbol in (A) and (B) represents an individual mouse. Error bars represent SEM. Statistical analyses were performed using unpaired Student's t tests. *p < 0.05.

RESULTS

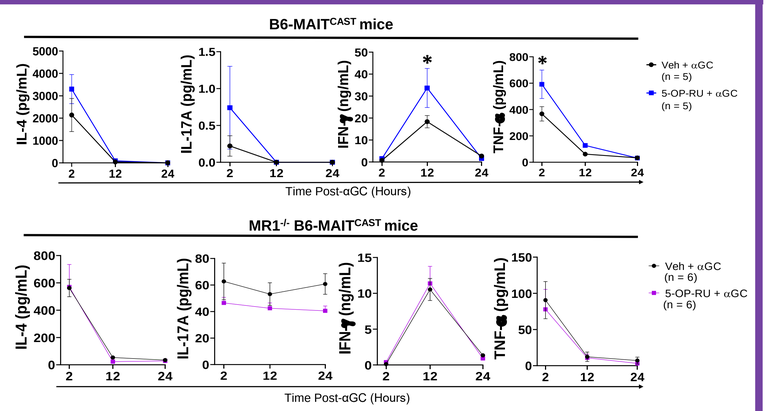


Figure 3. *In vivo* priming of MAIT cells with 5-OP-RU prior to *i*NKT cell stimulation with αGC results in increased serum IFN-γ and TNF-α levels. **(A)** B6-MAIT^{CAST} mice (n = 5) or MR1$^{-/-}$ B6-MAIT^{CAST} mice (n = 6) were injected i.p. with either vehicle or 5-OP-RU. Mice subsequently received αGC i.p. 24 hours later. Serum was then collected via a lateral saphenous vein bleed 2, 12, or 24 hours after the αGC injection. Serum IL-4, IL-17A, IFN-γ, and TNF-α levels were quantified by ELISA at the indicated timepoints. **(B)** Serum IL-4, IL-17A, IFN-γ, and TNF-α levels from MR1$^{-/-}$ B6-MAIT^{CAST} mice (n = 6) were quantified by ELISA at the indicated timepoints. Error bars represent SEM. Statistical analyses were performed using two-way ANOVA with Sidak's correction. *p < 0.05.

CONCLUSION

- ***In vivo* activation of iNKT cells results in an activated MAIT cell phenotype in murine lung and spleen (Figure 1).** Following iNKT cell stimulation, the frequency of CD69+ MAIT cells as well as the overall surface CD69 expression on per MAIT cell basis were both greatly increased.
- ***In vivo* MAIT cell activation did not appear to induce iNKT cell activation marker expression (Figure 2).** While we did observe a reduction in the hepatic iNKT cell population following MAIT cell activation, it remains unclear whether these iNKT cells are activated.
- **Priming MAIT cells prior to iNKT cell activation can lead to a skew towards a TH1-type cytokine profile in the serum of mice, as seen by increased TNF-α and IFN-γ levels (Figure 3).** This skew was absent in MAIT-deficient MR1$^{-/-}$ B6-MAIT^{CAST} mice, suggesting that MAIT cells are crucial for this phenomenon.
- Future work will look at assessing this cross-talk in murine disease models. Experiments involving human liver and blood samples will also be conducted to assess whether these findings apply to humans as well.

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