Letters

Crohn’s-associated invariant T cells (CAITs) recognise small sulfonate molecules on CD1d

MAIN

In the recent study by Rosati et al, we described a novel unconventional T cell population enriched in the peripheral blood of patients with Crohn’s disease (CD) and characterised by a semi-invariant T cell receptor (TCR) repertoire. However, the specificity of these Crohn’s-associated invariant T (CAIT) cells was not defined. Identifying the specificity of CAIT cells is essential to understand the origin of the antigen triggering their enrichment in CD.

In our previous study, we observed that CAIT cells have TCRs similar to those reported for some natural killer T (NKT) type II cells. Here, we performed a sequence similarity analysis and identified a large cluster composed of CAIT clonotypes and three reported NKT type II clonotypes (figure 1A). While the NKT type II and CAIT clonotypes all had highly similar TCR alpha chains carrying TRAV12-1/TRAJ6 genomic segments, their beta chains were highly diverse (figure 1A, bottom). Dash et al have shown that TCRs with similar sequences frequently have the same specificity. In the original publications describing these clonotypes, the authors reported that the NKT type II cells recognise small molecules of the pentamethylbenzofuransulfonates (PBFs).
family presented by the invariant HLA-like CD1d protein.\(^2\)\(^3\) Thus, we investigated whether CAIT TCRs shared the specificity of the NKT type II cells.

We transduced TCR-null NFT-AIT-GFP reporter Jurkat cells with constructs encoding two representative CAIT TCRs and two coclustering NKT type II TCRs (figure 1B). We synthesised two of the PBF compounds, phenyl-pentamethylidyldihydrobenzofuran-sulfonate (PBF), the original compound identified as a CD1d-dependent activator of NKT type II cells,\(^2\) and a more potent PBF analogue chlorophenyll-pentamethylidyldihydrobenzofuran-sulfonate (CIPBF)\(^2\) (figure 1C). Importantly, Jurkat cells naturally express CD1d and thus can act as antigen-presenting cells for CD1d-dependent antigens. To evaluate TCR activation, we cultured Jurkat cell lines with incremental concentrations of PBF and CIPBF (figure 1D).

All four transgenic cell lines (CAIT1, CAIT2, PNAS04, PNAS21) reacted to both compounds in a dose-dependent manner (figure 1E). Consistent with the original study, all tested TCRs reacted more strongly to CIPBF. The CAIT2 cell line, with the lowest level of activation, (figure 1E) carried the most dissimilar TCR alpha sequence compared with the other cell lines. CAIT2 has mismatches at CDR3 positions 4 and 7 (figure 1B), suggesting the importance of these positions for TCR avidity. A control cell line with known TCR specificity was not activated by the compounds, indicating that compound recognition is TCR-dependent (figure 1E). The reactivity of all cell lines dropped to undetectable levels in the presence of the CD1d-blocking antibody, demonstrating that the TCR interaction is CD1d-restricted (figure 1F).

To further investigate the possible role of NKT type II cells in CD, we searched all NKT type II sequences reported in Almeida et al in our previously published TCR data from patients with CD and healthy controls.\(^1\)\(^3\) Only TCRs from NKT type II cells with the characteristic CAIT TCRalphamain motif showed significant enrichment in patients with CD, while other TCRs were found in comparable amounts in patients and controls (figure 1G). Thus, only a subgroup of NKT type II cells with specific TCR features is enriched in CD.

While we show the specificity of CAIT cells for PBF small molecules in the context of CD1d, many questions remain. Many other small molecules similar to PBFs exist, including drug derivatives and microbial metabolites.\(^3\)\(^7\)\(^8\) It is thus reasonable to hypothesise that different small molecules may be triggering CAIT cells in vivo. Importantly, NKT cells can differentiate into opposing phenotypes, from proinflammatory to regulatory,\(^6\)\(^8\)\(^10\) necessitating further characterisation on the functional profile of the NKT type II CAIT cell subset and its behaviour in patients with CD.

Detailed methodologies are described in online supplemental file 1.

**Anastasia A Minervina,** 1 Mikhail V Pogorelyy, 1 Steffen Paysen, 2 Ulrich Luening,2 Frauke Degenhardt,2 Andre Franke,3 Paul G Thomas,1 Elisa Rosati 1,3,4

1Department of Immunology, St Jude Children's Research Hospital, Memphis, Tennessee, USA
2Otto-Diels-Institute for Organic Chemistry, Christian-Albrecht University of Kiel, Kiel, Germany
3Institute of Clinical Molecular Biology, University Hospital Schleswig Holstein, Kiel, Germany
4Institute of Immunology, Christian-Albrecht University of Kiel, Kiel, Germany

**Correspondence to** Dr Andre Franke, Institute of Clinical Molecular Biology, University Hospital Schleswig Holstein, Kiel, Schleswig-Holstein; a.franke@ikmb.uni-kiel.de or Paul G Thomas; Paul.thomas@STJUDE.de.

**Twitter** Elisa Rosati @elisarosix

**Contributors** Conceptualisation: ER, MVP, AAM; analysis: ER, MVP, AAM; Visualisation: AAM; Resources: PG, AF, FD, SP, UL; Supervision and coordination: AF, PG; ER. Writing original draft: ER, MVP, AAM; Funding: acquisition of Kiel, Germany

**Funding** This work was funded by American Lebanese Syrian Associated Charities (ALSAC) at St. Jude, SU01A1510747 (PGT), SR01A136514 (PGT). This work received infrastructure support from the EU's Horizon 2020 SYSCID program under the grant agreement No 733100 and the DFG Excellence Cluster Precision Medicine in Chronic Inflammation (EXC2167-390884018)

**Competing interests** ER is currently employed in the Institute of Clinical Molecular Biology, University Hospital Schleswig Holstein, Kiel, Schleswig-Holstein; a.franke@ikmb.uni-kiel.de or Paul G Thomas; Paul.thomas@STJUDE.de. All authors provided discussion, participated in revising the manuscript, and agreed to the final version.


Received 12 September 2022
Accepted 10 November 2022
Published Online First 25 November 2022

**ORCID iD** Elisa Rosati http://orcid.org/0000-0002-2635-6422

**REFERENCES**