AB009. Spatial proteomic analysis of the human thymic microenvironment shaping T cell development

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Background: The human thymus is essential for the generation of naïve T cells tolerant to self-antigens yet competent to recognise and respond to foreign antigens. This process of selection requires specific interactions of thymocytes with haematopoietic antigen-presenting cells (APCs), and diverse populations of thymic stromal cells. Previous studies have reported the frequency and gene expression profiles of different thymic cell types. However, spatial information detailing the location of these populations within the thymic microenvironment have largely been unavailable. This lack of spatial data precludes the identification of *in situ* cell interactions and their architectural organisation into functional niches.

Methods: To address this knowledge gap, we employed a multiplexed imaging method that co-detects 28 markers on a single tissue section of a 1-week-old human thymus. A custom image processing pipeline was developed to accurately segment and integrate the analysis of cells with both a regular round cell shape and with more complex irregular cell outlines. Using unsupervised machine learning approaches, individual cell phenotypes were identified and placed within the context of specific intrathymic regions.

Results: We identified and simultaneously mapped 33 cell-types, including thymocytes at various developmental stages, different APCs, and non-haematopoietic stromal cells that collectively formed 11 unique regions defined by spatial and compositional characteristics. The frequency, distribution, regional density, and morphological characteristics of individual cells were quantified and their cell-cell interactions determined. This analysis characterised specific cell niches and identified novel spatial interactions. Notably, we observed pre-emigrant CD4+ T cells interacting with endothelial cells in the inner but not outer cortico-medullary junction (CMJ). In contrast, pre-emigrant CD8+ thymocytes displayed a higher likelihood of interactions with endothelial cells of the outer CMJ, suggesting that mature naïve T cells select distinct routes of thymic egress dependent on their phenotype.

Conclusions: Our study provides novel insights into the thymic microenvironment and lays the groundwork for future research to understand the complex cellular mechanisms that underlie T cell development and the induction of central tolerance. We plan to apply the developed approaches to study abnormal thymic microenvironments, such as those associated with increased risk of developing autoimmune disorders, including thymic epithelial tumors.

Keywords: Thymopoiesis; tolerance; CODEX; spatial; hyperplex

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the North West-Haydock Research Ethics Committee (No.: 20/NW/0001) and individual consent for this retrospective analysis was waived.

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