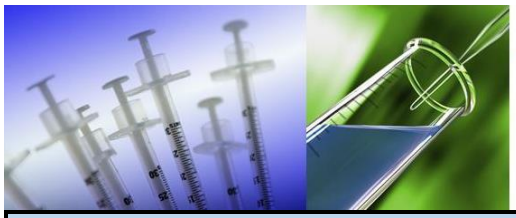
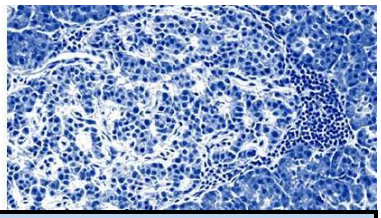

HANDEL-I - Cellular and Molecular Dynamics in the Developing Human Immune System

AUTHORS

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PURPOSE

Through the HANDEL project, we established a network to identify potential organ donors in the first decade of life, representing a very rare and largely understudied, yet exceedingly important, group for scientific investigation, this decade is characterized by massive growth and development affecting all organ systems, as well as the emergence of autoantibodies followed by the peak incidence of clinical type 1 diabetes (T1D). Recognizing that this period is also where environmental exposures, infections, and vaccines shape immune system development from central tolerance to cell subset distribution in peripheral lymphoid and mucosal tissues, we established HANDEL-I to collect these key tissues in parallel. This systems approach is only possible through the combined acquisition and analyses outlined in the overall HANDEL network.

METHODS

To date, we have procured 78 total donors, with distribution across four age categories (birth to < 3 months, n=16; ≥ 3 to < 24 months, n=20; ≥ 2 to < 5 years, n=19; ≥ 5 to < 11 years, n=23) This coordinated effort has allowed us to amass 77 spleens, 77 lymph nodes, 64 pancreata (with 16 islet isolations), 26 PBMC (blood cell isolates), 22 thymi, 24 lungs, 25 intestines, and 8 bone marrow samples across all age categories.

SUMMARY OF RESULTS

Herein, we discuss analyses of the central immune tissue – thymus and bone marrow.As the site of T cell development, the thymus plays a key role in immune ontology and pathogen defense. The developmental program of the thymus is highly dynamic, with a period of massive output following birth to near-total involution post-puberty. Studies of thymic development have been largely limited to animal models or cardiac surgery-associated thymectomy. To illuminate the tissue architecture and cellular dynamics of human thymic development, tissue sections obtained from two thymic regions incorporating both lobes were processed for comprehensive imaging by immunohistochemistry (IHC), highly multiplexed immunofluorescence imaging (CODEX), imaging mass cytometry, and clarity techniques (Lightsheet). Single cell suspensions generated by mechanical and enzymatic disruption were further interrogated by flow cytometry and single-cell RNA sequencing (scRNASeq).

Our preliminary sample cohort demonstrated dynamic changes in both structural and cellular architecture with age. Combined single-cell proteomics and transcriptomics via flow cytometry and scRNAseq facilitated immune cell type classification and the analysis of cell-fate trajectories from common progenitors to fully mature T cells prepared for thymic egress. Bone marrow not only serves as the source of hematopoetic stem cells (HSC), but in addition serves as a reservoir for long-lived memory and plasma B cells that are capable of supporting protective humoral immunity. As with the thymus, there are populations present that simply do not circulate at high levels in PBMC. Furthermore, very little is currently known about the amount of repertoire overlap amongst BM and PBMC. This outstanding need may very well be the determining factor for recurrent autoimmunity after beta cell replacement therapies, even when alloimmunity is well controlled. Our data, including scRNAseq, CITEseq, and immune repertoire analysis of T and B cells are expected to facilitate one of the first complete sets of data from both central developmental tissues including BM and thymus, along with mucosal immune sites, secondary lymphatics, and PBMC.

CONCLUSIONS

Our in-depth analyses of cellular architecture and transcriptional profiles provide novel insight for understanding human immune cell development in early life. The resulting knowledge offers the potential to uncover novel strategies to manipulate immune developmental checkpoints in early life, with substantial implications for autoimmune therapy strategies.