OP0233

CELLULAR SENESCENCE IN A MOUSE MODEL OF LUPUS NEPHRITIS

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Background: A third of patients with lupus nephritis (LN) show a decline in renal function despite treatment, with 5-10% developing end-stage renal disease within ten years. Prognostic markers that would allow for timely treat- ment decisions are hence eagerly sought, as are novel therapeutic targets. It is clear that lupus kidney is not simply a passive target of systemic autoimmunity but also hosts pathogenic mechanisms determining renal disease severity. These likely involve both the infiltrating immune and resident renal cell compartments. Cellular senescence is the irreversible arrest of the cell cycle through the accumulation of cyclin dependent kinase (CDK) inhibitors such as p16INK4a (CDKN2A). Senescent cells nevertheless remain metabolically active and undergo morphological and physiological changes including the acquisition of a pro-inflammatory, pro-fibrotic senescence-associated secretory phenotype (SASP). Aberrant accumulation of senescent cells has been observed in renal aging and pathology. We recently described the pres- ence of p16INK4a-positive cells (a senescence hallmark) in LN renal biopsies, and their association with baseline disease severity and 5 year outcome1. In addition, we observed a spatial co-distribution between tissue-infiltrat- ing CD8 T cells, senescent kidney cells, suggesting a pathogenic functional interaction between them.

Objectives: We hypothesize that cellular senescence may contribute to tissue damage in a few different ways: (a) Presentation of senescence-associated antigens that attract and activate CD8 T cells. Alternatively, CD8 T cells may be summoned to the kidney by other means, and may contribute to senes- cence- induction through the secretion of certain cytokines; (b) Secretion of pro-fibrotic, pro-inflammatory molecules, and/or (c) Functional incapacitation of kidney cells, particularly renal progenitor cells, responsible for repairing and restoring kidney function upon damage. In parallel with our work on patient samples, we aim to establish a relevant pre-clinical model in which we may test for the effects of senescence and senescence-directed interventions, on kidney damage.

Methods: Here, we assess for whether the B6.Sle1.Sle2.Sle3 spontaneous lupus-prone mouse may serve as an appropriate model in which to study the role of cellular senescence. We evaluated the presence and distribution of p16INK4a-positive cells by immunohistochemistry, and tested for an association with CD8 T cell infiltration and renal and systemic disease, in a cohort of 21 B6.Sle1.Sle2.Sle3 female mice. This is now being followed-up by a systematic, longitudinal study for the time of onset of different renal and systemic disease parameters, as compared to the detection of renal cell senescence in this well-characterized model.

Results: As observed in renal biopsies from LN patients, staining for p16INK4a-positive cells was heterogenous between mouse kidney samples. Interestingly, p16INK4a seems to be associated with CD8 T cell infiltration, renal impairment and damage, independently of age. This will now be confirmed using the "senescence-associated β-galactosidase" assay, the other classic measure of cellular senescence.

Conclusion: We report the occurrence of cellular senescence, and its corre- lation with CD8 T cell infiltration and disease severity, in the B6.Sle1.Sle2.Sle3 mouse model of lupus. These mice provide a pre-clinical model which we intend to use for the role of cellular senescence in the pathogenesis of LN in vivo (by the induc- tion vs. selective elimination of senescent cells). They also serve as an alterna- tive source (alongside patient samples) of cells for in vitro functional assays to test for the effects of senescent renal cells on CD8 T cells and vice versa.

REFERENCES:
[2] Disclosure of Interests: Gaelle Tillman: None declared, Laura Watteyne: None declared, Delphine Nolf: None declared, Caroline Bouzin: None declared, Frederic Housiaux Grantresearch support from: Grant from GSK, Nisha Limaye: None declared


OP0234

RNA-SEQ IN PERIPHERAL BLOOD IMMUNE CELLS IDENTIFIES MODULAR NETWORKS PREDICTIVE AND PROTECTIVE FOR PROGRESSION FROM ANA POSITIVITY TO CLASSIFIABLE SYSTEMIC AUTOIMMUNE DISEASE.

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Background: Anti-nuclear antibody (ANA) positivity represents a complex At-Risk state for progression of connective tissue disease (CTD). While ANA may become positive years in advance of clinically manifest CTD, they are also harboured in at least low titre by up to 25% of the wider population, of whom only a small fraction ultimately develop systemic autoimmunity. Complex immune dis- turbances including plasmacytoid dendritic cell exhaustion and non-haematopoi- etic interferon (IFN) production are evident even among ANA positive individuals who do not ultimately progress to connective tissue disease.[1]

In a prospective observational cohort of ANA positive individuals At-Risk for CTD we have shown that a validated blood IFN-Score was predictive of progression to classifiable SLE [2]. However, the wider transcriptional fingerprint of the At-Risk state and other factors modifying risk of progression are not known. We hypothesise that diverse immune processes, both independent and interacting with IFN pathway activation, could modulate disease progression.

Objectives: To investigate how peripheral blood immune cell transcriptional signatures derived by RNA Seq associate with progression or non-progression from At-Risk ANA positivity to clinically apparent CTD.

Methods: Peripheral blood mononuclear cells (PBMCs) were isolated at base- line from ANA-positive At-Risk individuals demonstrating ≥1 clinical criterion for classifiable CTD, symptom duration <12 months and naive of glucocorticoid or immunosuppressive therapy. Progression was prospectively adjudicated at 12 months and defined as accrual of clinical/ immunological criteria sufficient to meet classification for SLE (SLICC 2012) or other relevant CTDs. Bulk RNASeq was performed on PBMCs from 16 progressors and 19 non-progressors. Weighted gene co-expression network analysis (WGCNA) was performed using WGCNA package and gene ontology enrichment was evaluated using Clus- terProfiler, in R Bioconductor. The top 20% genes ranked by connectivity were defined as hub genes. Major cell subsets were quantified in parallel by multipara- meter flow cytometry.

Results: 29 modules were identified by WGCNA. Eigengenes for 3 modules were significantly associated with progression status. A single, 152 gene mod- ule showed strong positive correlation with progression (R=0.55, p<0.001). Hub genes were significantly enriched for type I IFN-signalling pathway and included established interferon stimulated genes such as IFI44 and IRF7. Two further modules had a negative, i.e. protective, association with progres- sion; a smaller 37 gene module, correlated negatively with both blood interferon score (R=-0.46, p=0.005) and with progression (R=-0.43, p=0.01). A larger 252 gene module was also negatively related to progression (R=-0.43, p=0.009) and demonstrated significant pathway enrichment for regulation of cell morphogene- sis and actin cytoskeleton organisation.

Conclusion: We identify novel modular transcriptomic signatures implicated in SLE disease initiation. We show (i) IFN-pathway activation is the single strongest transcriptomic risk marker of progression from the ANA positive At Risk state and (ii) we identify 2 novel protective signatures in peripheral blood immune cells for which further network-based characterization is ongoing.

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Disclosure of Interests: Lucy Marie Carter: None declared, Md Yusuf Consultant of: Aurinia Pharmaceuticals, Darren Plant: None declared, Adewoniu Alase: None declared, Jack Arnold: None declared, Antonios Psarr- ras: None declared, Zoe Wigston: None declared, Edward Vital Consultant of: AstraZeneca, Genentech, Aurinia, Lilly, ILTOO and Modus Therapeutics., Grant/ research support from: Astra Zeneca and Sandoz


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