Mass Cytometry Analysis Defines Clusters of SLE Patients with Differential Mucosal Phenotypes that Correlate with Immune Activation and Disease Severity

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Purpose

Systemic lupus erythematosus (SLE) is a prototypical systemic autoimmune disease characterized by immune tolerance breakdown and the production of autoantibodies. Although the precise mechanisms underlying individual clinical manifestations and prognosis in SLE are poorly understood, recent evidence revealed a dysregulation of mucosa-related immune populations in SLE. This highlights the need to investigate mucosa-related immunity as a potential source of patient heterogeneity.

Methods

To explore the role of mucosa-related immunity in SLE heterogeneity, we recently established a robust and scalable mass cytometry workflow for deep immune cell phenotyping and *ex-vivo* phosphorylation profiling of peripheral blood with two >50-plex antibody panels. We used a semi-automated data pre-processing and analysis pipeline to identify main immune lineages and subpopulations, and performed unsupervised clustering of patients based in the abundance of immune populations with a potential mucosal origin. Supervised analysis was performed to compare immune phenotypes and activation across patient groups and correlate them to clinical parameters

Results

We identified multiple lymphoid populations with a potential mucosal association present across SLE patients and healthy donors: namely IgA^{+} plasmablasts, a4b7^{+} CD4T cells, aEb7^{+} CD8T cells, mucosa-associated invariant T (MAIT) cells, gd T cells, and other subpopulations of double negative (DN) T cells. Next, we stratified SLE patients in 3 clusters with differential abundance of mucosal populations. Cluster 1 is primarily defined by an increased abundance of DN T cells and exhausted CD27- gdT cells, while cluster 3 is mainly driven by an increased abundance of a4b7^{+} CD4T and aEb7^{+} CD8T cells. As compared to cluster 1, patients belonging to cluster 3 show an elevated abundance of CD38+ CD21- DN B cells with increased pSTAT5 signaling, higher pSTAT3/5 signaling in naïve and memory CD4 and CD8 T cells and an increased abundance of cytotoxic Slamf7^{+} CD56^{high} NK cells. In the myeloid compartment, patients from cluster 3 showed increased phospho-signaling activity in monocytes and neutrophils (pCREB, pMAPK and pSTAT1). Clinically, patients in cluster 3 also showed an increased prevalence of renal involvement and higher SLEDAI-2K score and Physician Global Assessment.

Conclusion

Here, we describe differential mucosal signatures in SLE patients that correlate with immune activation, clinical manifestation and disease severity. Further work is required to elucidate the link between the changes observed in peripheral blood and potential alterations in the mucosal tissue immunity. To assess this, I will integrate blood phenotypes with microbiota phenotyping and sequencing data and correlate it with other paired data modalities (e.g. serum proteomics, stool metabolomics). Overall, our work suggests that mucosal immune dysregulation might be a relevant source of patient heterogeneity in SLE.