populations of CD16+ and CD14+ monocytes, and resident LYVE1+ and LYVE1- macrophages (figure 1).

Interestingly each infiltrating and residential cellular subset appeared to differentiate into these phagocytic macrophages in our trajectory analysis, suggesting that distinct cellular subsets converged on this common phagocytic state (figure 1). These phagocytic macrophages were infrequent in kidney biopsies collected from patients with non-autoimmune kidney disease (figure 3). Together, our findings suggest that phagocytic macrophages may play an important role in kidney remodeling and that these cells originated from distinct infiltrating and residential populations in response to kidney lesions found in lupus nephritis.

Abstract 2003 Figure 3 Phagocytic macrophages in lupus nephritis are infrequent in diabetic and hypertensive kidney disease. Fig. 3 Phagocytic macrophages (red outlines) and a small subset of LYVE1+ macrophages in lupus nephritis are infrequent in kidney biopsies from patients with diabetes and hypertension. Intrarenal myeloid cells from 155 lupus nephritis and 45 chronic kidney disease patients were integrated.

Background In recent years, our understanding of the mononuclear phagocyte system has expanded, highlighting previously unknown complexities in cell origin and function. However, to date few studies have examined a role for monocytes in tissues, with the majority of studies centered on circulating monocytes, or monocyte-derived macrophages. While transcriptional studies have exposed critical gene signatures for classical monocytes (CM) and non-classical monocytes (NCM) in the bone marrow and circulation, no such studies examined heterogeneity and function at the tissue level.

Methods We utilized functional genomic analysis of murine and human synovium including single-cell-CITE and ATAC-seq.

Results Here, we identify and characterize intravascular (i.v.) and extravascular (e.v.) synovial monocyte populations (Syn Ly6c- cells) which are distinct in surface marker expression and transcriptional profile from circulating monocytes, dendritic cells and tissue macrophages, and are conserved in in patients with rheumatoid arthritis. e.v. Syn Ly6c- cells are independent of NR4A1 and CCR2, long-lived and embryonically derived while the i.v. Syn Ly6c- cells are dependent on NR4A1, short lived and derived from circulating monocytes. e.v. Syn Ly6c- cells undergo increased proliferation and reverse diapedesis dependent on LFA1 in response to arthrogenic stimuli and are required for the development of inflammatory arthritis.

Conclusions These findings uncover a new facet of mononuclear cell biology and are imperative to understanding tissue-resident myeloid cell function in the synovium.

Transcriptomics

Gene expression profiling of key immune/inflammatory pathways reveals molecular endotypes of SLE with clinical implications

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Background SLE patients exhibit considerable clinical and molecular heterogeneity. A robust patient stratification approach can help to characterize individual lupus patients more effectively and aid patient care.

Methods We employed gene set variation analysis (GSVA) of informative gene modules and k-means clustering to identify molecular endotypes of SLE patients based on dysregulation of specific biologic pathways and interrogated them for clinical utility. We utilized machine learning (ML) of these molecular profiles to classify individual lupus patients into singular molecular subsets and used logistic regression with ridge penalization to develop a novel, composite metric estimating the severity of disease based on lupus-related immunologic activity. Shapley Additive Explanation (SHAP) was employed to understand the impact of specific molecular features on the patient sub-setting.

Results Six molecular endotypes were identified in a proof-of-concept cohort from the Illuminate trials (GSE88884) using baseline gene expression profiles. Significant differences in clinical characteristics were associated with different endotypes, with the least perturbed transcriptional profile manifesting the lowest disease activity, and endotypes with more perturbed transcriptional profiles exhibiting more severe disease activity. The more abnormal endotypes were also identified as more likely to have a severe flare over the 52 weeks of the trial and specific endotypes were more likely to be clinical responders to the investigational product (tablumab). GSVA and k-means clustering of 3166 samples in 17 datasets revealed a total of eight SLE molecular endotypes, each with unique gene enrichment patterns, but not all endotypes were observed in all datasets. ML algorithms were trained and validated on 2183 patients from GSE88884 (ILLUMINATE-1 and ILLUMINATE-2) and three additional datasets (GSE116006, GSE65391, and GSE45291) and demonstrated high degrees of accuracy (98%), precision (94%), sensitivity, and specificity in classifying patients into one of the eight endotypes. A composite molecular score, which comprised aggregate molecular scores of each GSVA gene module, was calculated for each lupus patient. A subset of patients was identified whose molecular scores were not different than those found in normal subjects, whereas other subsets of lupus patients had progressively higher scores indicative of the aggregation of molecular abnormalities. The composite molecular scores were significantly correlated with both anti-DNA titers and SLEDAL. Finally, SHAP analysis of the impact of input GSVA scores indicated that a unique array of features was influential in sorting individual samples into each of the molecular endotypes.

Conclusions Transcriptomic profiling and ML allowed for reproducible separation of lupus patients into molecular endotypes with significant differences in clinical outcomes and responsiveness to therapy.

Gene expression profiles were reduced to a score to assess lupus-related immune activity that correlated with clinical features, the implementation of which may provide a means to categorize lupus patients numerically based on the nature of each individual’s underlying molecular abnormalities.

Lay Summary Lupus patients present with arrays of symptoms that are highly variable, which we describe as heterogeneity. This heterogeneity is also present at a molecular level which means the biological mechanisms underlying disease differ from patient to patient at a given moment in time. We have addressed the clinical challenges presented by this heterogeneity by developing a new way to identify endotypes, or subsets of patients with commonalities in these underlying mechanisms. We used data from thousands of patients in multiple datasets to ensure we are representing the likely universe of lupus patients and used computational algorithms to not only subset the patients but also develop machine learning models that can accurately predict subset (endotype) membership. Finally, the underlying molecular commonalities among these subsets were simplified to the calculation of a single score reflecting an individual patient’s current status of immunologic perturbation. Together, these analyses should provide a new way to categorize lupus patients based on information not currently captured in clinical settings.