DEEP PROFILING

Elucidating the molecular circuitry of autoimmunity

Novel single-cell profiling technologies have delineated the cellular and molecular landscapes that dominate the joints in rheumatoid arthritis and the skin and kidneys in systemic lupus erythematosus, shedding light on potential pathogenic mechanisms.

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The cellular and molecular complexity present within the inflamed end organs in systemic autoimmune diseases remains poorly understood, partly due to limited access to these tissues and partly because of limits in technology. This scientific barrier has been breached with the advent of newer single-cell profiling technologies. Three reports in this issue of *Nature Immunology* now detail the cellular and molecular landscapes that dominate the skin, joints and kidneys in two of the most common and complex of the systemic autoimmune diseases: systemic lupus erythematosus and rheumatoid arthritis (RA)[1–3]. All three studies adopt a similar strategy (broadly outlined in Fig. 1).

Flow cytometry has long served as the standard for high-throughput investigation of cellular suspensions and enables the identification of cell types by immunofluorescence labeling. Flow cytometry can process thousands of cells per second, but the number of unique proteins that are detectable is generally only ~10–20 per experiment. The development of mass cytometry that utilizes immuno–mass labeling has increased the number of unique proteins detected to ~30–50 per experiment[4]. Due to the combinatorial nature of protein expression, that increase, in turn, has led to much deeper profiling of cell types. In parallel, the development of next-generation sequencing has facilitated large-scale sequencing, and with the aid of sufficient amplification it is possible to estimate the abundance of mRNA molecules at the single-cell level[5]. Since there is usually a positive correlation between transcript abundance and protein expression, at least for differentially expressed genes, the single-cell RNA-sequencing (RNA-seq) data can be used to directly infer the expression of differentially expressed proteins and create atlases of cells that constitute the different anatomical compartments.

The two accompanying reports on lupus nephritis (LN), by Der et al.[1] and Arazi et al.[2], use single-cell RNA-seq analysis, focusing predominantly on resident tubular epithelial cells and infiltrating leukocytes, and add several novel insights to this field. Together they reveal the unexpected complexity of almost all leukocyte lineages that become greater in abundance within LN kidneys. While re-affirming the elevation in the abundance of follicular helper T cells and age-associated B cells within LN kidneys (relative to their abundance in healthy kidneys), they also identify an unanticipated prominence of $CD8^+$ T cells and natural killer cells within the LN kidneys. They shed light on cell types that have heightened expression of an interferon signature as well as pro-fibrotic signatures and on their respective associations with treatment response. Also emphasized is the relationship between hyper-expressed pathways in tubular epithelial cells and treatment response in LN. Most intriguingly, they highlight the correlation of gene signatures in keratinocytes with those in renal tubular epithelial cells, as well as the correlation of gene signatures expressed by intra-renal cells with gene signatures associated with cells present in the urine.

These studies also provide evidence for the putative transition from inflammatory blood monocytes to phagocytic macrophages, and then to alternatively activated macrophages, as well as transitions from naive B cells to eventual age-associated B cells within the renal milieu in LN, based on 'trajectory analysis'[1,2]. Interesting insights also emerge that suggest that increased expression of the chemokine receptor CXCR4 and the cytokine receptor TNFRSF10A (tumor-necrosis factor receptor superfamily member 10A) on cells infiltrating the LN kidney immune cells results in engagement of their ligands (CXCL12 and TNFSF10 (TRAIL), respectively) on tubular epithelial cells. Similar interactions between innate immune cells and adaptive immune cells within LN kidneys achieved via other ligands, are also suggested. Notably, traditional approaches could not have provided the same

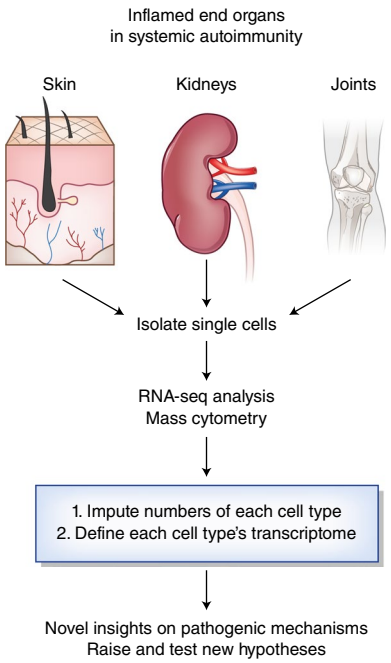

Fig. 1 | Harnessing the power of single-cell profiling to study end-organ inflammation. Three studies in this issue of *Nature Immunology* have isolated single cells from various target organs commonly inflamed in systemic autoimmune diseases and have profiled them at single-cell resolution through the use of RNA-seq analysis and other complementary techniques[1–3]. This rich dataset has allowed the imputation of the various cell types that populate the various target organs. In this fashion, the authors are able to achieve fairly comprehensive detailing of the cellular and molecular landscapes that dominate the joints in RA and the skin and kidneys in LN.

information presented in these studies, at such a level of comprehensiveness. Although independent validation with additional samples and further examination of additional resident cell types are warranted, these clearly represent a substantial advance in the understanding of the cellular and molecular makeup of LN. The ability of cells in the urine to recapitulate the molecular changes within nephritic kidneys also lends itself to future biomarker studies, since urine is readily accessible.

RA is the most common autoimmune disease and is estimated to affect ~1% of the world's population. RA is associated with chronic inflammation in the synovium of the joints that leads to joint destruction and a reduced lifespan. Although multiple groups have previously profiled each of the cellular compartments within the synovium separately, in the accompanying article, Zhang et al. use a collection of techniques (population-level RNA-seq analysis, flow and mass cytometry, immunofluorescence staining, and single-cell RNA-seq analysis) on samples from a cohort of 36 patients with RA and compare those results with results obtained for samples from 15 patients with osteoarthritis[3]. In order to perform deep profiling of the various cell compartments, the authors use flow cytometry to sort separate populations of synovial T cells, B cells, monocytes and fibroblasts, which they then subject to the various assays to determine cell state and composition.

One of the challenges associated with such multimodal data is the ability to integrate the data; the authors use canonical correlation analysis to enable pairwise matching of datasets. In simple terms, canonical correlation analysis maximizes the correlation between the two sets of variables. Like principal-component analysis, it generates two new sets of variables that arise from a linear combination of variables within each set such that the correlation between these two new sets is maximal. This approach allows a reduction in the number of variables and preserves the relationships present in the two original sets of variables.

Flow cytometry–based assessment of the frequency of infiltrating leukocyte subsets and comparison to the same populations in osteoarthritis identifies leukocyte-rich RA that, unsurprisingly, is associated with much greater inflammation. Comparative cross-data assessments are used to define subsets of populations among each of the cell types studied: aggressive fibroblast populations with the potential to secrete pro-inflammatory cytokines such as IL-6; pro-inflammatory monocytes capable of releasing the cytokine IL-1β; pathological peripheral helper T cells, including effector $CD8^+$ T cells characterized by upregulation of the cytokine IFN-γ and granzymes; and B cells with signatures consistent with those of autoimmune B cells. The extensive network of pro-inflammatory cytokines is indicative of cross-regulation between stromal cells and the immune compartment in RA. As outlined by the authors, these results present the first map of the subsets of the cell types that are associated with RA progression. As with the accompanying studies of lupus[1,2], this study[3] represents a pivotal advancement; however, more comprehensive validation, with profiling of patient-specific cell-subset composition, profiling of the end organs earlier in disease before irreversible damage sets in and association of changes in end organs to clinical disease, is warranted.

Of particular note, the insights gained from all three studies[1–3] are facilitated by advanced single-cell profiling. With all three studies, the availability of these novel signatures at single-cell resolution will allow other researchers and clinicians to deconvolute population-level RNA-seq data to identify the composition of cellular subsets in RA, systemic lupus erythematosus and other autoimmune diseases. These findings will also allow researchers to formulate new hypotheses and plan future studies to test these hypotheses. Understanding of the molecular events that mediate disease within the targeted end organs in autoimmune diseases has always lagged behind the understanding of such events in systemic disease. However, the advent of emerging single-cell profiling platforms promises to bridge this gap. ❐

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Competing interests

The authors declare no competing interests.

HIV IMMUNITY

A SNP of lncRNA gives HIV-1 a boost

Although the molecular basis of most disease-associated single-nucleotide polymorphisms has remained elusive, an HIV-1 viral load–associated polymorphism (rs1015164) has been identified that marks expression of a long non-coding RNA that regulates the co-receptor CCR5 and thereby influences infection of $CD4^+$ T cells.

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The chemokine receptor CCR5, which belongs to the G-protein–coupled receptor family, is liganded by several inflammatory cytokines, including CCL3, CCL4 and RANTES, and serves as a co-receptor for the entry of human immunodeficiency virus type 1 (HIV-1) into $CD4^+$ cells. Polymorphisms in or near *CCR5* have been correlated with differences in HIV-1 disease progression and other viral infections[1]; however, the molecular basis for many of these associations has not been elucidated. In this issue of *Nature Immunology*, Kulkarni et al. report a novel mechanism of post-transcriptional regulation of *CCR5* by a long non-coding RNA (lncRNA)[2] and thus provide an explanation for a single-nucleotide polymorphism (SNP) linked to the outcome of infection with HIV-1 (refs. [2,3]).