Molecules and Cells

Minireview

Single-Cell Genomics for Investigating Pathogenesis of Inflammatory Diseases

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Recent technical advances have enabled unbiased transcriptomic and epigenetic analysis of each cell, known as "single-cell analysis". Single-cell analysis has a variety of technical approaches to investigate the state of each cell, including mRNA levels (transcriptome), the immune repertoire (immune repertoire analysis), cell surface proteins (surface proteome analysis), chromatin accessibility (epigenome), and accordance with genome variants (eQTLs; expression quantitative trait loci). As an effective tool for investigating robust immune responses in coronavirus disease 2019 (COVID-19), many researchers performed single-cell analysis to capture the diverse, unbiased immune cell activation and differentiation. Despite challenges elucidating the complicated immune microenvironments of chronic inflammatory diseases using existing experimental methods, it is now possible to capture the simultaneous immune features of different cell types across inflamed tissues using various single-cell tools. In this review, we introduce patient-based and experimental mouse model research utilizing single-cell analyses in the field of chronic inflammatory diseases, as well as multi-organ atlas targeting immune cells.

Keywords: autoimmune disease, chronic inflammatory disease, coronavirus disease 2019, mouse model, multi-organ atlas, single-cell genomics

INTRODUCTION

Unbiased profiling of single cells from inflamed status allows understanding of immune networks between heterogeneous cell types. Although single-cell profiling technology, such as flow cytometry, has been used to evaluate the protein expression of a few dozen antigens, recent technical advances have enabled unbiased transcriptomic and epigenetic analysis of each cell. In this context, the human cell atlas, the most comprehensive reference map of the molecular state of cells, was established based on high-throughput single-cell profiling (Regev et al., 2017). Immunologists have actively used single-cell genomics studies to rapidly and comprehensively reveal the pathogenesis of coronavirus disease 2019 (COVID-19) in the recent pandemic era (Lee et al., 2020; Stephenson et al., 2021; Unterman et al., 2022). In this review, we introduce recent progress in understanding inflammatory diseases by applying single-cell genomics technologies and propose new directions for future studies.

CURRENT SINGLE-CELL TOOLS FOR IMMUNOLOGICAL STUDIES

Single-cell analysis offers a variety of windows by which to observe the state of each cell, including cell surface proteins, intracellular proteins, mRNA levels, DNA methylation, genome sequence, chromatin accessibility, and histone modifications (Stuart et al., 2019). Exploring the immune system,

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these integrative tools can be applied to measure multiple immune cell characteristics not only in the transcriptome, but also in the genome, methylome, and proteome.

Single-cell immune repertoire analysis

V(D)J recombination constructs highly diverse repertoires of antigen receptors in T cells and B cells, which is important for the development of adaptive immunity (Schatz and Ji, 2011). Compared to the early T cell receptor (TCR) analysis methods, which can capture partial sequences, the advent of NGS (next-generation sequencing) and single-cell analysis allowed the capture of paired TCR$\alpha\beta$ chain information at single-cell resolution with transcriptome data (Pai and Satpathy, 2021). With the development of immune profiling, TCR sequencing has been applied to study the immunological responses of COVID-19 patients, showing that a diverse TCR repertoire was absent in patients with severe conditions (Zhang et al., 2020). In line with prior studies, robust TCR clonal expansion and less B cell receptor (BCR) clonal expansion was found in peripheral blood mononuclear cell (PBMC) samples from asymptomatic COVID-19 patients compared to moderate and severe patients (Zhao et al., 2021). Using LIBRA-seq to map the antigen specificity of B cells in high-throughput at single-cell levels (Setliff et al., 2019), a transition from immunoglobulin M (IgM) to IgG was observed in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-specific and cross-reactive B cells in response to BNT162b2 vaccination, suggesting an improvement in SARS-CoV-2 antibody specificities and cross-reactivity after vaccination (Kramer et al., 2022). Apart from COVID-19 studies, Piper et al. (2022) reported that $CD4^+$ GM-CSF^+ IFN-γ^- T cells possess a distinct TCR repertoire compared to other $CD4^+$ clusters, unraveling their heterogeneity in gastrointestinal tract GVHD (graft-versus-host disease) (Piper et al., 2022). The number of studies investigating TCR or BCR repertoires in human subjects is growing rapidly, but the diversity of TCR or BCR sequences is still considerably larger than the scale of current commercial single-cell genomics platforms.

Single-cell surface proteome analysis

Immunophenotyping with flow cytometry (fluorescence-activated cell sorting [FACS]) has been the principal method of identifying immune cell subsets by staining surface markers or intracellular cytokines (Perfetto et al., 2004). Using oligonucleotide-labeled antibodies, cellular indexing of transcriptomes and epitopes by sequencing (CITE-seq) can identify protein markers with unbiased transcriptome profiling at the single-cell level. CITE-seq can detect separate pools based on the CD8a fluorescence of flow cytometry, indicating its applicability in immunophenotyping using single-cell transcriptomics (Stoeckius et al., 2017). Considering that CITE-seq has no optical limitations, future proteomics analyses will be able to include higher dimensions than FACS.

Single-cell epigenome analysis

Post-translational modification of chromatin, such as DNA and histone methylation, is dynamic and changes during the development and differentiation of cells. These modifications, which are connected to chromatin accessibility, reflect how certain genomic regions function in regulating gene expression (Turner, 2007). First applied to the single-cell platform by Buenrostro et al. (2015) and Cusanovich et al. (2015), single-cell assay for transposase-accessible chromatin sequencing (ATAC-seq) has become a well-liked and straightforward method for profiling chromatin accessibility spanning thousands of individual cells (Minnoye et al., 2021). Global chromatin remodeling has been observed in the epigenomic profiles of SARS-CoV-2 patients using single-cell ATAC-seq, which promotes the activation and differentiation of monocytes and SARS-CoV-specific effector and memory $CD8^+$ T cells (You et al., 2021). Extensive analysis of chromatin accessibility and gene expression has identified a unique T_H1-like pathogenic T_H17 program shared by pT_H17 cells and T_H1 cells and discovered BACH2 as a novel regulator of T_H17 pathogenicity (Thakore et al., 2022). Nevertheless, single-cell ATAC-seq requires nucleus extraction before generating libraries, and combining TCR and/or BCR immune repertoires can be challenging.

Single-cell expression quantitative trait loci (eQTLs)

Single-cell eQTL overcame the pre-existing limitations of eQTL with bulk RNA sequencing (RNA-seq) which represents average gene expression in selective cell types or tissues. By connecting the single-cell level of gene expression with disease-associated genomic variant information provided by GWASs (genome-wide association studies), researchers can better investigate eQTL effects based on the dynamic states of individual cells. Yazar et al. (2022) discovered 990 trans-eQTL effects outside the MHC locus and identified 305 loci contributing to autoimmune disease based on transcriptomic changes in specific cell types. To better depict the plasticity and diversity of memory T cells, Nathan et al. (2022) focused on continuous transcriptional landscapes when dissecting eQTL effects at single-cell resolution. They demonstrated that continuous multimodally defined cell states were responsible for most of the cis-eQTL effects, indicating the importance of cell-state context in understanding eQTL pathogenicity. Context-specific gene regulation was identified and related to immune disease-associated variants in $CD4^+$ T cells in accordance with prior studies (Soskic et al., 2022).

COVID-19 AS A MODEL DISEASE

After a new coronavirus known as SARS-CoV-2 appeared in the Chinese city of Wuhan in 2019, COVID-19 rapidly spread worldwide and presented a severe danger to global public health (Hu et al., 2021). As of September 18, 2022, more than 637 million confirmed COVID-19 cases and 6.62 million deaths have been reported worldwide (https://ourworldindata.org/). Single-cell analysis has been an effective tool for investigating robust immune responses in COVID-19, capturing diverse, unbiased immune cell activation and differentiation. Since Spring 2020, there have been more than 200 publications on single-cell analysis of COVID-19 (Liu et al., 2022a). COVID-19 serves as a disease model that enables the improvement and development of single-cell analytic methods and technologies.

Based on a large set of data from patients, researchers

have constructed a dynamic immune cell atlas of COVID-19. Large-scale single-cell RNA-seq of PBMCs, bronchoalveolar lavage fluid, and sputum in 196 COVID-19 patients revealed upregulated transcriptomic levels in interferon-stimulated genes (ISGs) of SARS-CoV-2-infected epithelial and immune cell types (Ren et al., 2021). Levels of inflammatory genes, such as *S100A9* and *S100A8*, are also increased in the monocytes and megakaryocytes of patients, contributing to "cytokine storms". Expansion of nonclassical monocytes, megakaryocyte-committed progenitors, and effector $CD8^+$ T cells have been identified by single-cell multi-omics analysis, including immune profiling and CITE-seq (Stephenson et al., 2021).

Approximately 20% of COVID-19 patients have an exacerbation of severe diseases (Wu and McGoogan, 2020), followed by a hyperinflammatory response and immune dysfunction. Our group performed single-cell RNA-seq in patients with mild COVID-19, severe COVID-19, or severe influenza (Lee et al., 2020), finding distinctive upregulation of tumor necrosis factor (TNF)/interleukin (IL)-1β-driven inflammation and the type I interferon (IFN) response in PBMCs from severe COVID-19 patients compared to others, indicating a significant role in hyperinflammation in severe COVID-19. In severe COVID-19 patients with acute respiratory distress syndrome, the transition of plasmablasts to neutrophils with type I IFN-driven inflammatory signatures and human leukocyte antigen (HLA) class II downregulation of monocytes were observed by cellular trajectory analysis (Wilk et al., 2020). Single-nucleus RNA-seq of the lungs of patients who died from COVID-19 has shown dense infiltration of activated macrophages, transition of alveolar type 1 cells to type 2 cells, and *CTHRC1*$^+$ pathological fibroblasts, exacerbating pulmonary fibrosis in COVID-19 (Melms et al., 2021).

COVID-19 has been the subject of many immunological studies, but the main issue is preparation for the next pandemic. It is essential to maintain a system of effective research on treating critically ill patients with inflammatory diseases whose vital signs are affected by unknown infectious diseases.

LARGE-SCALE COHORT-BASED APPROACHES TO CHRONIC INFLAMMATORY DISEASES

The intricate immune microenvironments of chronic inflammatory diseases have been difficult to study with existing methods, which are insufficient to distinguish the heterogenic transcriptional changes in myriad types of cells that participate in inflammation. However, with the advent of high-resolution technologies, unbiased single-cell analysis-based approaches can capture the simultaneous immune features of different cell types across inflamed tissues. In Table 1, we summarize the studies that have used single-cell genomics to investigate the pathogenesis of chronic inflammatory diseases (Table 1).

Ankylosing spondylitis (AS)

AS is a chronic inflammatory disease featuring inflammation at the spine and sacroiliac joints with bone remodeling and ankylosis (Mauro et al., 2021). Applying single-cell RNA-seq and ATAC-seq on PBMCs from AS patients, *NFKB* expression and TNF signaling pathways have been shown to be upregulated in $CD8^+$ T cells with abnormal accessibility of *FOS, JUN*, and *JUNB* (Xu et al., 2021). This finding suggests the role of effector $CD8^+$ T cells in inflammatory cytokine secretion, including TNF-α. Single-cell CITE-seq has shown that *TNFSF10* and IL-18Rα are overexpressed by $CD16^+$ monocytes and cytotoxicity-related genes are upregulated in effector memory $CD8^+$ T cells and NK cells (Alber et al., 2022). In immunological studies, Th17 cells have primarily been studied in terms of their pathological role in AS, but recent single-cell transcriptome or epigenome studies suggest major roles of other immune cell populations, including $CD8^+$ T cells. Interactions between specific immune cell subpopulations need to be revealed to explain different features of inflammation in AS.

Inflammatory bowel diseases (IBDs)

IBD is characterized by chronic inflammatory processes of the digestive tract and comprises Crohn's disease (CD) and ulcerative colitis (UC). CD is characterized by patchy transmural inflammation occurring at the distal parts of the small intestine (up to 80%) and colon (up to 30%), whereas UC is associated with persistent superficial inflammation in the colon (Neurath, 2019). Jaeger et al. (2021) observed an increase in activated T_H17 cells compared to other T cell subsets in CD patients' intraepithelial lymphocytes and lamina propria. GIMATS module, consisting of IgG plasma cells, inflammatory mononuclear phagocytes, activated T cells, and stromal cells was defined in a subgroup of large ileal CD cohorts by mapping the ileal lamina propria using single-cell analysis (Martin et al., 2019). Moreover, GIMATS module scores are increased in CD patients with anti-TNF resistance. In UC, pro-inflammatory resident memory $CD8^+$ T cells have increased expression of *EOMES*, which is known as the master regulator of $CD8^+$ effector and memory T cells (Boland et al., 2020).

Multiple sclerosis (MS)

MS is a chronic inflammatory neurological disease that causes demyelination and neurodegeneration. Various MS-associated features, including responses to antigens and IL-17-producing capacities, are widely recognized to be related to diverse innate and adaptive immune cells (Attfield et al., 2022). For example, cytotoxic-like helper T cells, follicular helper T cells (T_{FH}), and B lineage cells are increased in the CSF of MS patients, supporting the efficacy of B-cell-depleting therapies (Schafflick et al., 2020). Because CNS (central nervous system)-resident inflammatory immune cells are mainly infiltrated from peripheral blood, Kaufmann et al. (2021) performed single-cell RNA-seq and CITE-seq on PBMCs from MS patients with a relapsing-remitting disease course (RRMS). They discovered unique CNS-homing $CD4^+$ T cells that promote white matter demyelination and are situated in the cortical brain, causing progression to disability in RRMS. To profile the ongoing inflammatory environment of demyelinated white matter lesions, single-cell nucleus sequencing with magnetic resonance imaging identified 'microglia inflamed in MS' (MIMS), with complement component 1q (C1q) as a significant mediator of MIMS (Absinta et al., 2021).

Table 1. Current single-cell analysis-based studies on chronic inflammatory diseases

Inflammatory disease	Species	Tissue	Single-cell analysis techniques	Reference
Ankylosing spondylitis	Human	PBMC	Transcriptome, epigenome	(Xu et al., 2021)
	Human	PBMC	Transcriptome, surface proteome analysis	(Alber et al., 2022)
	Human	PBMC, SFMC	Transcriptome, immune repertoire analysis	(Simone et al., 2021)
Inflammatory bowel diseases	Human	Intestinal epithelium, lamina propria from terminal ileum resections	Transcriptome	(Jaeger et al., 2021)
	Human	Lamina propria from ileum, PBMC	Transcriptome	(Martin et al., 2019)
	Human	Rectum, PBMC	Immune repertoire analysis	(Boland et al., 2020)
	Human	Colonic biopsies	Transcriptome (both single cell and spatial)	(Garrido-Trigo et al., 2022)
Multiple sclerosis	Human	Cerebrospinal fluid, PBMC	Transcriptome	(Schafflick et al., 2020)
	Human	Post mortem brain tissue, PBMC	Transcriptome (both single cell and spatial), epigenome	(Kaufmann et al., 2021)
	Human	Post mortem brain tissue	Transcriptome	(Absinta et al., 2021)
	Mouse	$CD11b^+$ myeloid cell from spleen	Transcriptome	(Lu et al., 2020)
	Mouse	MOG-specific $CD4^+$ T cells	Transcriptome	(Krienke et al., 2021)
	Mouse	Memory-phenotype $CD4^+$ T cells	Transcriptome, immune repertoire analysis	(Cho et al., 2022)
Chronic inflammatory skin diseases	Human	Skin biopsies	Transcriptome, epigenome	(Liu et al., 2022b)
	Human	Skin biopsies	Transcriptome	(Nakamizo et al., 2021)
	Human	Skin biopsies	Transcriptome	(Kim et al., 2021)
	Human	Skin biopsies	Transcriptome (single cell and spatial)	(Schäbitz et al., 2022)
Rheumatoid arthritis	Human	Synovial biopsies	Transcriptome	(Zhang et al., 2019)
	Human	Synovial biopsies, PBMC	Transcriptome	(Wu et al., 2021)
	Human	Synovial fluid, PBMC	Immune repertoire analysis	(Argyriou et al., 2022)
	Human	Synovial biopsies	Transcriptome	(Edalat et al., 2022)
	Mouse	$CD4^+$ T cells from lymph node, spleen	Immune repertoire analysis	(Ashouri et al., 2022)
Systemic lupus erythematosus	Human	PBMC	Transcriptome	(Nehar-Belaid et al., 2020)
	Human	PBMC	Transcriptome	(Guo et al., 2022)
	Human	PBMC	Transcriptome, eQTLs	(Perez et al., 2022)
	Mouse	B cells from spleen	Transcriptome, immune repertoire analysis	(Zheng et al., 2022)
Primary Sjögren's syndrome	Human	PBMC	Immune repertoire analysis	(Hou et al., 2022)
	Human	PBMC	Transcriptome	(Hong et al., 2021)
	Human	Labial minor salivary gland biopsies	Transcriptome (both single cell and spatial)	(Nayar et al., 2022)
	Mouse	Submandibular gland	Transcriptome	(Horeth et al., 2021)
Type 1 diabetes mellitus	Human	Pancreatic islet, PBMC	Transcriptome, epigenome	(Chiou et al., 2021)
	Human	Pancreatic islet	Transcriptome	(Fasolino et al., 2022)
	Mouse	Pancreatic islet	Transcriptome	(Zakharov et al., 2020)

PBMC, peripheral blood mononuclear cell; SFMC, synovial fluid mononuclear cell; MOG, myelin oligodendrocyte glycoprotein; eQTLs, expression quantitative trait loci.

Chronic inflammatory skin diseases

Chronic inflammatory skin lesions commonly occur in two immune diseases: atopic dermatitis (AD) and psoriasis vulgaris (PV). Different from allergic features of T_H2-related AD, PV are defined by the key pathological mediator IL-23 and IL-17 secreted by T_H17 and T_H1 cells (Armstrong and Read, 2020). Performing single-cell RNA-seq and CITE-seq on the sorted $CD45^+$ cells from AD and PV skin biopsies, transcriptomic differences in resident memory T cells (T_{rm}) from AD and PV were discovered in accordance with previous findings (Liu et al., 2022b). Nakamizo et al. (2021) analyzed myeloid lineages in the skin of PS patients and identified a novel subtype of dendritic cells and its role, which is IL-1β and IL-23A producing $CD14^+$ type 3 dendritic cells, promoting simultaneous inflammations in PS. Semimature dendritic cells and type 17 T cells expressing IL-23A and IL36G have been found in unbiasedly harvested samples of PS skin biopsies (Kim et al., 2021).

Rheumatoid arthritis (RA)

One of the most common autoimmune diseases worldwide, RA is a chronic inflammatory joint disease characterized by the infiltration of the synovial membrane of joints by immune cells, leading to bone erosion and cartilage degradation (Aletaha and Smolen, 2018; Ryu et al., 2019). Using specific immune-related cell types sorted from the synovial tissues of patients with RA and osteoarthritis, Zhang et al. (2019) performed a comprehensive analysis combining single-cell RNA-seq, FACS, mass cytometry, and bulk RNA-seq. They observed increased *THY1*$^+$ *HLA-DRA*^{hi} fibroblasts, *IL1B*$^+$ inflammatory monocytes, *PDCD1*$^+$ peripheral helper T cells (T_{PH}), $T_{FH,}$ and *ITGAX*$^+$ *TBX21*$^+$ autoimmune-associated B cells in RA patients (Zhang et al., 2019). Because RA can be categorized by the presence of anticitrullinated-peptide antibodies (ACPAs), Wu et al. (2021) studied the differences in the immunopathogenesis of $ACPA^+$ and $ACPA^-$ RA and discovered decreased *HLA-DRB5*$^+$ B cells and upregulated *CCL13, CCL18,* and *MMP3* expression by myeloid cells in $ACPA^-$ RA patients. Based on these discoveries, they reclassified RA subtypes as $ACPA^-$ with inflammatory myeloid cells and $ACPA^+$ with lymphoid cells according to the key cell types that contributed to the disease. Similar to the prior study, single-cell RNA-seq of the synovial $CD4^+$ T cells from both $ACPA^+$ and $ACPA^-$ RA patients identified clonal expansion of $GPR56^+$ $LAG3^+$ $CXCL13^{high}$ T_{PH} in $ACPA^+$ RA, which was confirmed by flow cytometry (Argyriou et al., 2022). However, RA has not yet been reported to be caused by a single pathogenic subset of immune cells. A larger cohort including RA patients with heterogenous immunological features is needed to depict diverse clinical aspects.

Systemic lupus erythematosus (SLE)

SLE is a systemic autoimmune disease across the skin, joints, CNS, and kidneys. Antinuclear antibody production and the preponderance in childbearing-age females are well-known clinical characteristics of SLE (Kaul et al., 2016). Nehar-Belaid et al. (2020) discovered high expression of ISGs in most immune cell subtypes among the PBMCs of 33 young SLE patients. They also discovered associations between ISG-expressing cell populations and high disease activity in adult cohorts. Because dysfunctional Treg cells mainly contribute to various autoimmune diseases (Dominguez-Villar and Hafler, 2018), upregulated exhaustion-related gene expression, which is similar to ISG, has been identified in $CCR7^{low}$ $CD74^{hi}$ Treg subgroups from SLE patients (Guo et al., 2022). Perez et al. (2022) profiled PBMCs in a large SLE cohort and showed elevated ISG expression in monocytes, decline in naïve $CD4^+$ T cells, and expanded repertoire-restricted cytotoxic *GZMH*$^+$ *CD8*$^+$ T cells. They also performed single-cell eQTL and discovered that elevated IFN levels can modify the genetic effects of cis-eQTLs, highlighting the pathological role of IFN in the SLE microenvironment.

Primary Sjögren's syndrome (pSS)

pSS is a systemic autoimmune disease with dryness of mouth and eyes, fatigue, and joint pains, which are caused by infiltration of the exocrine glands by lymphocytes (Mariette and Criswell, 2018). Analyzing the PBMCs of pSS patients by single-cell immune profiling, Hou et al. (2022) found differentially expressed genes in multiple immune cell subtypes but did not capture any significant changes in the TCR repertoire. Expansion of $CD4^+$ cytotoxic lymphocytes has been identified with chemokine receptor gene expression and upregulated type 1 IFN-related genes across immune cell types in the PBMCs of pSS patients (Hong et al., 2021). Nayar et al. (2022) performed single-cell RNA-seq and spatial transcriptomics to better understand the pathological role of tertiary lymphoid structures (TLS) in the minor salivary glands of pSS patients. They found that immunofibroblasts and stromal cells expressing *ACKR3* and *CD55* are key components of TLS formation in salivary glands, supporting immune cell migration and survivability.

Type 1 diabetes mellitus (T1DM)

T1DM is a chronic autoimmune disease with loss of the pancreatic islet β-cells leading to insulin deficiency and hyperglycemia (Katsarou et al., 2017). By performing single nuclei ATAC-seq to identify candidate *cis*-regulatory elements (cCREs), T1DM variants were discovered to be broadly enriched in T cell cCREs (Chiou et al., 2021).

ANIMAL MODEL-BASED APPROACHES TO CHRONIC INFLAMMATORY DISEASES

Despite its significance in clinical applications, using patient samples to study autoimmune and chronic inflammatory diseases has limitations. Firstly, unlike patients with infectious diseases, which begin with exposure to pathogens, it is difficult to know the starting point of chronic inflammatory diseases. Secondly, patients have genetically diverse backgrounds and studies have limitations in using cross-sectional samples. Thirdly, with successive developments in clinical approaches to autoimmune diseases, it has been difficult to observe certain cases that progress in severity without treatment. Animal model-based approaches have the advantages of carefully controlled studies, and it is easier to configure immune mechanisms by using certain genetically engineered mice. Because there are various animal models designed to exhibit immune responses similar to humans (Jeon and Choi, 2016), we are going to introduce widely used experimental

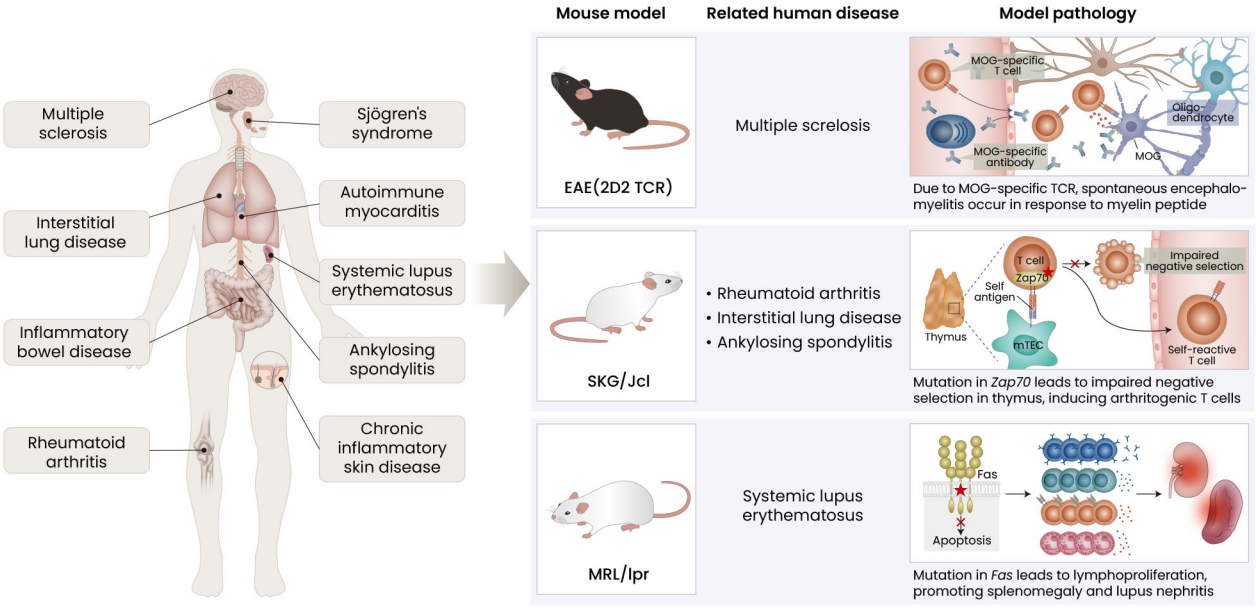

Fig. 1. Animal model-based approaches to chronic inflammatory diseases in human patients. EAE, experimental autoimmune encephalomyelitis; TCR, T cell receptor; MOG, myelin oligodendrocyte glycoprotein; mTEC, medullary thymic epithelial cell.

models in the field of chronic inflammatory and autoimmune diseases (Fig. 1).

Experimental autoimmune encephalomyelitis (EAE)

EAE is a commonly used mouse model for MS. However, the model is induced by myelin antigens with adjuvants, which is not similar to MS pathology of non-specified antigens (Constantinescu et al., 2011). Producing a conditionally *Stat3*-deleted mouse in the EAE model to discover the role of Stat3 in MS lesion-infiltrating monocytes, Lu et al. (2020) showed downregulated expression of genes involved in the innate immune response, antigen presentation and processing, cytokine, and inflammatory responses in the $CD11b^+$ cells of *Stat3*-deleted mice. Krienke et al. (2021) applied nanoparticle-formulated 1 methylpseudouridine-modified messenger RNA (m1 Ψ mRNA) in the EAE model to induce immune tolerance by $CD11c^+$ dendritic cells as a vaccine. Surprisingly, they observed a significant surge in effector Treg cells followed by upregulated expression of immunosuppressive genes, whereas the populations of inflammatory T_H1, T_H17, and $T_H1/17$ cells were reduced compared to control groups.

SKG mouse model

With a recessive point mutation of ZAP-70 on the BALB/C genetic background, the SKG/Jcl mouse features spontaneous development of $CD4^+$ T cell-mediated chronic autoimmune diseases, including RA (Sakaguchi et al., 2003). The SKG mouse model has also been widely used to study other types of chronic inflammatory diseases, including AS and interstitial lung disease (Jeong et al., 2017; Shiomi et al., 2014). Ashouri et al. (2022) established a Nur77-eGFP reporter SKG mouse (SKG^{Nur} mouse) to identify antigen-reactive T cells. With single-cell RNA-seq and immune profiling, they identified a cluster of naïve $CD4^+$ T cells with upregulated TCR signaling ($T.N4_{Nr4a1}$). $T.N4_{Nr4a1}$ cells from the SKG^{Nur} mouse model have an increased number of especially early activated subgroups (*Egr* module) with a biased TCR variable beta gene repertoire, defining arthritogenic naïve $CD4^+$ T cells.

MRL/LPR mouse model

MRL/LPR mice have defects in *Fas* expression, which interfere with negative selection during thymic development of T cells, triggering the escape of autoreactive T cells. Due to this, MRL/LPR mice are prone to lymphadenopathy and systemic autoimmune disease—not only SLE, but also other autoimmune diseases (Watanabe-Fukunaga et al., 1992). Zheng et al. (2022) generated *Ezh2* conditional knockout in $CD19^+$ cells from MRL/LPR mice and performed single-cell RNA-seq and BCR seq. They discovered downregulation of *XBP1* in B cells, which led to decreased B cell development, autoantibody production, and improved glomerulonephritis.

Non-obese diabetic (NOD) mouse model

NOD/ShiLtJ mice have a genetic susceptibility related to the A^{g7} MHCII allele and multiple non-MHCII genes affecting T-cell function, which leads to the destruction of insulin-producing β cells in islets by lymphocyte infiltration (De Riva et al., 2013). The NOD mouse is widely used not only for studying T1DM, but also Sjögren's syndrome (Park et al., 2015). Defining the longitudinal stage of T1DM, memory $CD4^+$ and cytotoxic $CD8\ T^+$ cells with proinflammatory-polarizing islet macrophages have been observed in the pancreatic islets of NOD mice (Zakharov et al., 2020).

MULTI-ORGAN ATLAS OF INFLAMMATION

Represented by RA and SLE, some chronic inflammatory diseases feature symptoms related to systemic inflammation in multiple organs. Approximately 25% of patients with autoimmune diseases develop additional autoimmune diseases (Cojocaru et al., 2010), indicating the need for a comprehensive multi-organ and multi-timepoint atlas of immune responses for chronic inflammatory diseases.

To distinguish tissue-specific features of immune cells, Dominguez Conde et al. (2022) performed single-cell RNA-seq and immune profiling on immune cells from up to 16 organs from healthy donors. They developed CellTypist, an extensive reference database of immune cell types and annotated 15 major populations and 43 minor subtypes of T cells, B cells, ILCs, and mononuclear phagocytes. In the context of T cells, they discovered resident memory T cells (T_{RM}) that produce IFN-γ/IL-17A, though with tissue restriction of clonal expansion. Apart from immune cells, but related to our topic, Korsunsky et al. (2022) analyzed tissue-resident fibroblasts in four different chronic inflammatory diseases (RA, inflammatory bowel disease, interstitial lung disease, and Sjögren's syndrome) and identified two distinct fibroblasts across all tissues: $CXCL10^+$ $CCL19^+$ immune-interacting and $SPARC^+$ $COL3A1^+$ vascular-interacting fibroblasts, which expanded under inflammatory conditions. Multi-organ single-cell transcriptomic atlas studies have been performed by Tabula Muris Consortium et al. (2018), Tabula Sapiens Consortium et al. (2022), and Gökcen Eraslan et al. (2022) that also feature immune cells and immune-related cells, such as fibroblasts.

However, few studies are available to show the application of single-cell analysis in a mouse and human multi-organ atlas targeting immune cells. Our group is currently constructing one of the single-cell level atlases of a chronic arthritis model.

CONCLUDING REMARKS

Single-cell genomics data associated with immunological diseases is primarily focused on uncovering rare or unique cell types, which are defined by transcriptome. The next step will be to determine their pathological roles in inflammatory diseases. Though genetically engineered animal models may provide confirmation of the pathological roles of newly discovered cell types, cohort studies including relevant patient specimens will provide real-world evidence to develop new therapeutic strategies. Larger size, longitudinal sampling from initial diagnosis, and multi-organ sampling would be required to establish a human atlas of chronic immune diseases. Integrating transcriptome, TCR/BCR, epigenetic, and proteomic data will be increasingly important to develop answers to immunological questions in inflammatory diseases.

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AUTHOR CONTRIBUTIONS

S.J. and J.S.L. conceived idea, wrote the manuscript, and secured funding.

CONFLICT OF INTEREST

The authors have no potential conflicts of interest to disclose.

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