



Synovial Tissue: Cellular and Molecular Phenotyping

Sara. Shanaj¹ · Laura T. Donlin^{1,2}

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Abstract

Purpose of Review This review provides a summary of recent molecular findings that have refined our understanding of the cell types that constitute human synovial tissue, particularly in patients with rheumatoid arthritis (RA).

Recent Findings Recent advances in high-dimensional and single-cell assays have elucidated upwards of 20 cell subsets in the RA synovium. This includes novel fibroblast populations and lymphocyte phenotypes, which in many cases exhibit features that have not been found in other tissues thus far.

Summary Molecular profiling studies over the past several years have rapidly generated a comprehensive and detailed outline of the cellular phenotypes in synovial tissue affected by RA. Molecular features of these newly identified cell subsets immediately represent reasonable therapeutic targets and provide the opportunity to design the most clinically relevant mechanistic experiments. Broadly speaking, the ~20 cell types thus far identified in RA synovium seem to be fairly well conserved across patients, despite extensive heterogeneity in patient clinical features, stage of disease, and treatment responses. Thus, a next phase in molecular profiling may benefit from quantifying patient samples in terms of the ratios of cell types, with the rationale that certain cellular interactions will predominate in an individual and medications targeting these interactions may be more efficacious for that individual. Such cellular profiling in tissues combined with studies examining how the compendium of these cells interact in their three-dimensional tissue ultrastructures will be important in understanding how collectively these cells drive the disease process and ultimately how best to treat patients.

Keywords Rheumatoid arthritis · Synovial tissue · Autoimmune disease · Synovial fibroblasts · Fibroblast-like synoviocytes

Introduction

Synovial tissue encapsulates articular joints and homeostatically maintains synovial fluid for smooth and efficient motion [1]. Directly interfacing with the synovial fluid, the synovial intimal lining is formed from spindle-shaped fibroblasts that align in a cohesive layer. Unlike mucosal surfaces, there is no basement membrane in the synovial intima, wherein synovial fibroblasts are less stabilized than epithelial cells with their tight junctions.

Macrophages are interspersed within this fibroblast layer, and together, these cells produce and turnover the synovial fluid. In the subintima, the extracellular matrix predominates, with fibroblasts, macrophages, adipocytes, and vasculature speckled throughout (Fig. 1a).

In the autoimmune disease rheumatoid arthritis (RA), the synovial tissue is marked by an extensive infiltration of lymphocytes, which form spherical aggregates adjacent to blood vessels (Figs. 1b and 2). The synovial fibroblast compartment expands in large numbers, leading to tissue remodeling with papilla formation that extend beyond the natural tissue borders, which can ultimately invade and damage cartilage and bone [2].

Over decades, molecular approaches have successfully identified various cell types in RA synovial tissue, however, largely one cell type at a time. Developments in next-generation sequencing methods and other technologies that capture orders of magnitudes of more data points than prior molecular assays have provided the capacity to simultaneously study all cell types within a sample, including rare cell types with no established markers

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✉ Laura T. Donlin
donlinl@hss.edu

¹ Arthritis and Tissue Degeneration Program and the David Z. Rosensweig Genomics Research Center, Hospital for Special Surgery, New York, NY 10021, USA

² Weill Cornell Medical College, New York, NY 10021, USA

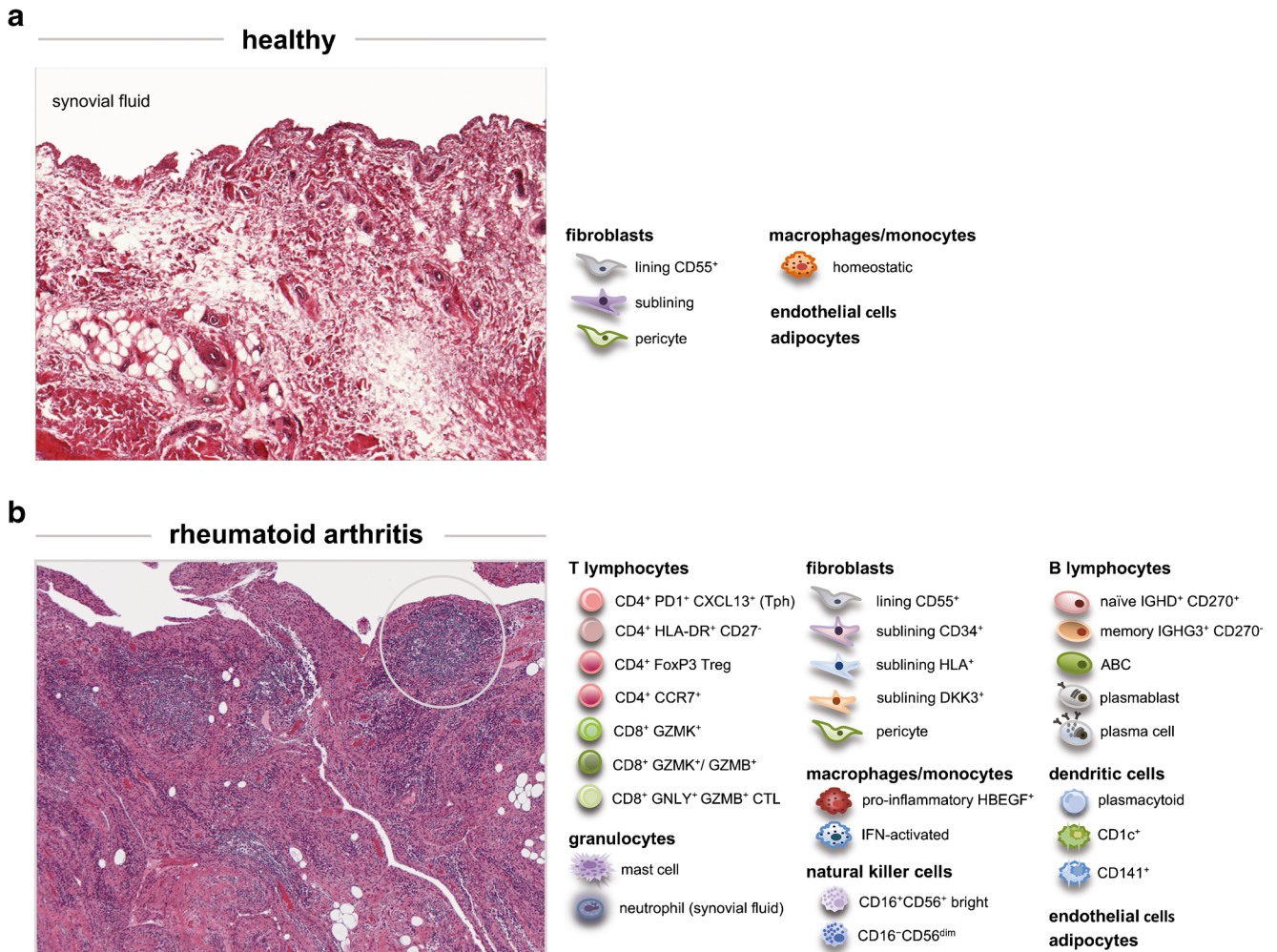
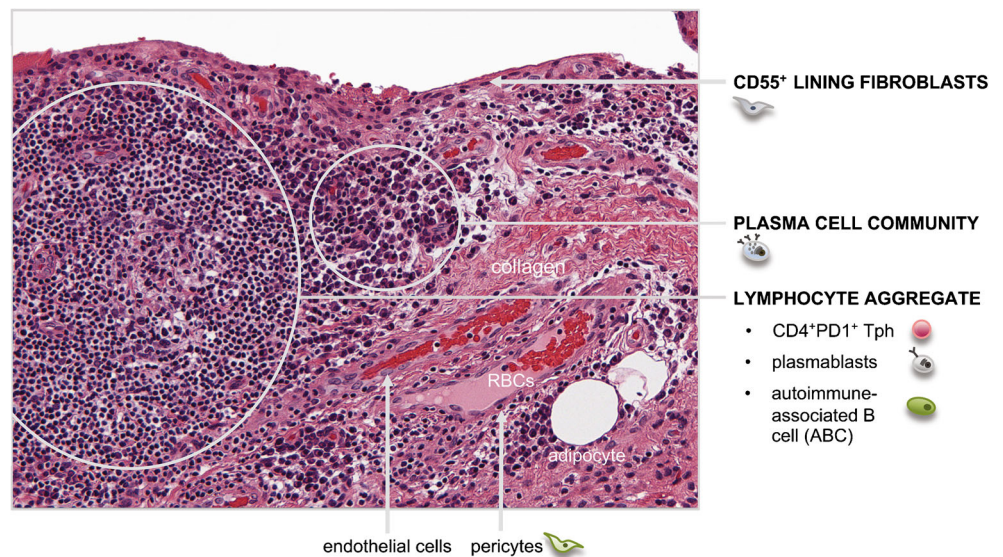


Fig. 1 An updated compendium of synovial cell subsets in RA. **a** Healthy synovium largely has been defined by synovial fibroblasts, macrophages, adipocytes, and cells of the vasculature and lymphatics. **b** Recent single-

cell and high-dimensional molecular findings have defined more than 20 unique cell subsets in RA synovium 5-fold magnification, image from H&E stained synovium

Fig. 2 Spatial localization of cell subsets in the RA synovium. A high magnification of inflamed RA synovial tissue found within the white circle on Fig. 1b. Cell types with established localization patterns are denoted. (RBCs: red blood cells).



[3]. Furthermore, these technologies can gain vast data on each individual cell, including transcriptome-wide gene expression profiles, thereby providing a considerably richer view of cellular activation pathways and phenotypes.

Identification of New Synovial Cell Subtypes

Synovial Cell Compendiums

Two recent publications in particular have provided an unprecedented and comprehensive view of cells found in RA synovial tissue using single-cell RNA-sequencing (scRNA-seq). In an unbiased analysis of dissociated tissues, Stephenson et al. [4•] identified 13 unique cell types across 5 RA patient samples. This included three unique fibroblasts subsets, five T and B cell subtypes, two myeloid populations, NK, and mast cells. The RA network within the Accelerating Medicines Partnership (AMP) consortium identified 18 unique cell subsets on more than 20 RA tissues, using single-cell and bulk RNA-seq of sorted cell types and integrating these findings with mass cytometry (CyTOF) data from the same tissues [5•, 6•]. Notably, despite considerable clinical differences across the RA patients in these two studies, the cell types did not differ dramatically, indicating to some extent a conservation of cell types across patient samples. Studies with larger cohorts will be important to firmly establish whether these cell types can be considered a reference framework from which quantitative and finer qualitative differences in cell compositions may be used to associate molecular and cellular features with clinical heterogeneity or differences in the response to a particular type of medication.

The following sections provide detailed information on the cell types described within these two compendium-style reports alongside other recent reports focused on a particular cell type.

Synovial Fibroblasts

While typically classified as stromal support cells in most tissues, fibroblasts in the synovial intima may also be reasonably classified as synovial parenchyma considering their specialized function in producing and encapsulating synovial fluid, arguably the main function of synovium [1]. In joint pathologies such as RA, considerable evidence has emerged that implicate synovial fibroblasts as active participants in perpetuating pathology [7, 8]. Thus, a deeper understanding of the impact of disease-associating fibroblast phenotypes and the impact of a presumed loss in their homeostatic roles has become an important research direction in RA, and various rheumatic diseases with stromal tissue pathology [9].

While a limited number of previously identified markers were available (e.g., *Cadh11*, *CD55*) [10, 11], scRNA-seq on synovial fibroblasts readily identified substantial differences in lining versus sublining fibroblast gene expression programs

[4•, 5••, 12•]. Intimal lining fibroblasts express high levels of genes consistent with synovial fluid production, such as *PRG4* (proteoglycans) and *HAS1* (hyaluronan), and also surface markers such as *CD55* that spatially map these fibroblasts to the intima (Fig. 1, *CD55*⁺ lining fibroblasts) [4•, 5••, 12•]. In the sublining, the three fibroblast subsets identified thus far in RA tissues exhibit consistently higher *CD90/Thy-1* surface protein in comparison with the lining *CD55*⁺ fibroblasts. Two of the sublining fibroblasts are highly enriched in RA tissues—including a population expressing high levels of *CD34* and a second with considerable MHC class II/HLA expression, an unusual observation outside of antigen-presenting cells of the hematopoietic lineage [5••]. Considering these findings, determining where precisely these fibroblast subsets localize in the sublining, how they impact RA pathophysiology, and how medications could be used to beneficially modify their activity has now become an important and attainable pursuit.

Macrophages and Monocytes

Macrophages, as highly dynamic cells, are considered pivotal in a variety of synovial tissue functions, including the turnover of synovial fluid, tissue repair processes, and antimicrobial immune protection in the joint [1]. Recent studies have looked to detail synovial macrophage phenotypes in arthritic conditions. Using synovial biopsies from RA patients, Mandelin et al. [13•] identified gene expression modules in macrophages that correlate with clinical features. Furthermore, the AMP consortium identified four distinct phenotypes of *CD14*⁺ synovial cells, two of which are highly enriched in the RA synovium [5••].

The most prominent of the RA-associated macrophage subtypes aligns neither with the classical pro- nor anti-inflammatory polarization phenotypes. Instead, these macrophages are marked by a unique expression pattern of growth factors and a select subset of pro-inflammatory genes (Fig. 1b, pro-inflammatory *HBEGF*⁺ macrophages). Kuo et al. [14•] determined that this RA-associated phenotype results from exposure of synovial fibroblast-produced prostaglandin *PGE*₂ and inflammatory cytokines such as TNF. The EGF ligands produced by these macrophages promote invasive activity of synovial fibroblasts, thereby potentially contributing to outgrowth of synovial tissue and invasion into cartilage and bone. Treating synovial tissue from RA patients with non-steroidal anti-inflammatory (NSAIDs or COX Inhibitors), which blocks the production of *PGE*₂, drove these macrophages towards a classic pro-inflammatory polarization state, potentially suggesting these medications inhibit only some arms of RA tissue inflammation. A second RA-enriched synovial *CD14*⁺ cell type was identified by the AMP consortium and exhibited a strong IFN response signature (Fig. 1b) [5••].

One of the monocyte/macrophage phenotypes defined by the AMP consortium was found in higher ratios in OA

synovial tissue and was marked by various genes involved in phagocytosis (Fig. 1, NUPR⁺ macrophage) [5••]. Enhanced phagocytic activity in OA synovial macrophages was indicated in a separate recent report and represents a difference in cellular function that may impact the distinct pathophysiology found in RA versus OA [15•].

Dendritic Cells

Dendritic cells (DC) coordinate both adaptive and innate immune responses. As rare cell types, however, locating and studying their function in primary human tissues poses a challenge. Nonetheless, upon cell sorting primary synovial cells with flow cytometry, one of the two main classical DC subsets marked by CD141⁺ cell surface expression was isolated and shown to be transcriptionally and functionally distinct in comparison with peripheral blood CD141⁺ cells [16•, 17]. The authors also observed reduced CD141⁺ DC in the blood of patients with inflammatory arthritides, specifically RA and psoriatic arthritis, and suggested this may indicate increased infiltration of these DC into the inflamed joints [16•].

T Cell Subsets

Infiltrating CD4⁺ T lymphocytes have long been documented as a hallmark of RA synovial tissue pathology and are directly implicated in RA disease by the strong genetic association of HLA-DR alleles. Using mass cytometry on synovial and blood samples, a T follicular-like helper cell population was recently identified as a heavily enriched lymphocyte population in the synovium of patients with RA. These CD4⁺PD1⁺CXCL13⁺ T cells, referred to as T peripheral helpers (Tph), are proposed to locally support B cell activation and antibody production in the synovium (Figs. 1 and 2) [18•]. This Tph phenotype has also now been observed on autoreactive T cells in the gut and blood of patients with celiac disease, indicating this may represent an autoreactive T cell phenotype common to disparate autoimmune conditions [19•]. A second CD4⁺ T cell subset identified by mass cytometry displays a cytotoxic-like effector memory phenotype with a CD4⁺HLA-DR⁺CD27⁻ surface profile. Additional CD4 T cells identified in the RA synovium by the AMP consortium using single cell and mass cytometry include T regulatory T cells (Tregs CD4⁺FOXP3⁺) and CD4⁺CCR7⁺ central memory-like phenotype [5••]. Of note, these recent reports did not identify CD4⁺ Th17 subsets in RA synovium.

For CD8⁺ T cells, at least three unique subsets have been identified that can be differentiated based on their unique expression profile of four granzyme genes (including *GZMK*, *GZMB*, *GZMA*, and *GNLY*) and a cytotoxic T lymphocyte (CTL) transcriptional signature in one subset (Fig. 1b). The CD8⁺ T cells in the synovium of patients with RA appear thus

far to be the dominant producers IFN γ , along with lower levels produced by CD4⁺ T cell subsets [6•].

B Cell Subsets

Considering autoantibody levels, in particular anti-citrullinated peptide antigen (ACPA) and rheumatoid factor (RF), are one of the few objective measures of RA disease; B cells have long been the focus of research in RA. After sorting CD19⁺ B cells from the synovium and analyzing them using scRNA-seq, the AMP consortium identified 4 subsets of synovial B cells: (1) naive IGHD⁺CD27⁻, (2) memory IGHG3⁺CD27⁻, (3) autoimmune-associated B cells (ABC) with high expression of *ITGAX* (CD11c) and *TBX21* (T-bet), and (4) a plasmablast cluster expressing high levels of IgG, along with XBP1, a transcription factor required for plasma cell differentiation (Fig. 1b) [5••]. Histologically, one can observe elevated plasma cell presence in highly inflamed RA tissue, but the use of CD19⁺ marker for isolation, which is significantly reduced in plasma cells, lowers the expected recovery of these cells. Plasma cells were identified in a separate single-cell RNA-seq study, in which all synovial cell types were analyzed without pre-sorting. Notably, at least two types of immunoglobulin-expressing plasma cell populations were identified, IgG⁺ and IgA⁺ plasma cells [4•].

NK Cells

NK lymphocyte subsets have also now been identified in the synovium of patients with RA. Single-cell analyses identified two unique NK populations: CD16⁺CD56^{bright} and CD16⁻CD56^{dim}, the latter subset identification reconfirming a prior study showing enrichment of CD16⁺CD56^{bright} NK cells in the synovium of patients with RA [4•, 20].

Additional Cell Types

The vasculature, lymphatics, and the neovasculature in inflammatory arthritis have long been appreciated as critical to the hyperplastic process in RA synovium. Recent reports have been limited in furthering our understanding of these structures at the molecular level, but undoubtedly will see important discoveries in the coming years with various technologies, including high-dimensional histology techniques and organoid studies particularly as the function of endothelial cells and pericytes, for example, is inherently dependent on vessel ultrastructure.

Adipocytes and mast cells are found in both the healthy and RA synovium (Fig. 1); however, a clear indication of how pathology impacts these cells in the joint awaits further inspection.

Conclusion

The last several years have witnessed a considerable leap forward in understanding the cells found in human synovia, particularly in RA pathology. New avenues to pursue include how these newly identified cell types differ in ratios across disease states, across patients with the same condition, and across different types of joints (consistent with differing gene expression patterns recently documented [21]). Certainly, the burgeoning capabilities in spatial transcriptomics and high-dimensional histology assays will enrich a three-dimensional understanding as to where each cell type is located, and which cell types interact. Lastly, the cellular compendium now detailed for RA synovium should be a launching point from which we can compare and subgroup patients and define how their composition of synovial cells reflects pathways that dominate in their disease and may be used to predict which medications would be most efficacious for their condition.

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Data Availability The Zhang et al. AMP consortium synovial dataset can be accessed in a visualization tool or in raw downloadable files at <https://immunogenomics.io/cellbrowser/>, and https://portals.broadinstitute.org/single_cell/study/amp-phase-1.

Compliance with Ethical Standards

Conflict of Interest Authors have nothing to disclose.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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