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Epigenetics and tissue immunity—Translating environmental cues into functional adaptations*

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Abstract

There is an increasing appreciation that many innate and adaptive immune cell subsets permanently reside within non-lymphoid organs, playing a critical role in tissue homeostasis and defense. The best characterized are macrophages and tissue-resident T lymphocytes that work in concert with organ structural cells to generate appropriate immune responses and are functionally shaped by organ-specific environmental cues. The interaction of tissue epithelial, endothelial and stromal cells is also required to attract, differentiate, polarize and maintain organ immune cells in their tissue niche. All of these processes require dynamic regulation of cellular transcriptional programmes, with epigenetic mechanisms playing a critical role, including DNA methylation and post-translational histone modifications. A failure to appropriately regulate immune cell transcription inevitably results in inadequate or inappropriate immune responses and organ pathology. Here, with a focus on the mammalian kidney, an organ which generates differing regional environmental cues (including hypersalinity and hypoxia) due to its physiological functions, we will review the basic concepts of tissue immunity, discuss the technologies available to profile epigenetic modifications in tissue immune cells, including those that enable single-cell profiling, and consider how these mechanisms influence the development, phenotype, activation and function of different tissue immune cell subsets, as well as the immunological function of structural cells.

KEYWORDS
chromatin accessibility, DNA methylation, kidney, macrophage, tissue immunity

1 | INTRODUCTION

Tissue immune responses require the coordinated activation of specialized cell types, both professional immune cells and organ structural cells, each with differing, but tightly regulated, transcriptional programmes that are switched on and off in response to pathogen or tissue damage signals. The precise immune functionality of these tissue populations is determined by the selection of genes they express, encoding proteins that differentially contribute to immune responses. Tissue immune cells encounter an array of organ-specific conditions

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that determine tissue-specific transcriptional programmes. Gene expression is controlled, in part, by the state of chromatin, with closed structures sterically hindering the binding of transcriptional modulators. Post-translational modifications of histone proteins, such as methylation or acetylation, alter chromatin compaction and accessibility, while DNA methylation of CpG dinucleotides may also influence gene expression by preventing transcription factor binding. The extent and nature of these epigenetic modifications are shaped by the stimuli encountered by cells, for example, engagement of toll-like receptor (TLR) 4 on macrophages by lipopolysaccharide (LPS) can alter subsequent gene expression upon re-challenge, due to changes in histone acetylation. In addition, several non-coding RNAs provide an additional layer of post-transcriptional control of gene expression. Regulation of immune cell transcriptional activity is paramount to ensure appropriate immune responses are generated and terminated in a timely manner, and a failure to do so may have negative consequences for organ homeostasis. Therefore, understanding the precise epigenetic mechanisms that control tissue immune responses will inform treatment strategies for a variety of diseases. Here, with a focus on the mammalian kidney, we will review the basic concepts of tissue immunity, discuss the technologies available to profile epigenetic modifications in tissue immune cells, and consider how these mechanisms influence the development, phenotype and function of different tissue immune cell subsets, as well as the immunological function of structural cells in health and disease.

2 | TISSUE IMMUNITY

2.1 | Tissue immunity—a coordinated effort by structural cells and tissue-resident immune cells

The study of mammalian immunity has historically focused on interrogating the responses of immune cells in blood or secondary lymphoid organs (lymph node and spleen). However, it is increasingly appreciated that several subsets of innate and adaptive immune cells reside in non-lymphoid organs.[1-3] These tissue-resident populations may constitute a large proportion of the total immune cell pool, and do not enter the circulation, permanently occupying a specific niche within tissues.[4] The archetypal tissue-resident cell type is the macrophage, exemplifying the canonical features of tissue-resident cells, being long-lived, self-renewing, and showing tissue-specific transcriptional and functional specialization.[5] Macrophages take up residency in tissue niches early in embryogenesis, seeding from the yolk sac (YS), and then fetal liver precursors.[6] Post-natally, macrophage tissue pools are variably replenished by monocyte-derived cells,[7,8] with tissue-specific cues, for example from the microbiome[8] (in the case of the intestine) or high interstitial sodium[9] (in the case of the kidney), influencing this process. Other prevalent tissue-resident immune cell subsets include T cells; antigen-specific $CD8^+$ T and $CD4^+$ T cells enter tissues during viral challenge, and persist long after the resolution of infection.[10-12] A common tissue-residency transcriptional signature has been described in lymphocytes,[13,14] and other tissue-resident subsets including innate lymphocytes and natural killer (NK) cells have also been characterized.[15,16] Tissue-resident immune cells play a variety of important functional roles in addition to immune defense, frequently contributing to organ homeostasis.[17-19] For example, human yolk sac-derived macrophages in the heart were physically connected to cardiomyocytes via gap-junctions containing connexin 43, which allows macrophages to participate in and regulate electrical conduction[20]; in the colon, muscularis macrophages regulate peristalsis.[21]

Effective tissue immune responses require the coordinated interaction of these resident populations with each other and with their circulating counterparts, via cytokine and chemokine production, as well as cross-talk with the epithelial compartment.[22-25] Indeed, immune functionality within organs is not limited to immune cells, but non-immune tissue cells can also play a part. For example, in human and mouse kidney, we previously showed that pelvic epithelial cells express antimicrobial peptides (AMP), directly contributing to anti-bacterial immunity, as well as producing neutrophil-recruiting chemokines, orchestrating the specific anatomical localization of the key circulating phagocytes to protect the kidney from bacteria ascending from the bladder.[26] Krausgruber et al[27] described the expression of immune mediators, as well as cytokines and chemokines in epithelium, fibroblast and endothelium, in a variety of mouse organs, generating so-called 'structural immunity'. Thus, tissue immune responses involve the combined efforts and interactions of epithelial, endothelial, stromal, and resident immune cells, and are tailored to the tissue-specific challenges encountered, requiring tissue-specific cues to control cellular transcriptional programmes.

2.2 | Experimental identification of tissue immune cells

Since all solid organs contain a vascular network, required to supply oxygen and remove metabolic waste, a major challenge in the field of tissue immunity has been how to accurately distinguish cells that are *bona fide* tissue-dwelling cells, from those that are in the circulation. In murine studies, three approaches have been applied to establish the tissue-resident status of an immune cell. Firstly, parabiosis,[28] in which the circulatory systems of two mice expressing a congenic surface marker (most often CD45.1 versus CD45.2) are surgically joined. After a number of weeks, tissue-resident populations remain donor-derived, whereas an equal number of donor (eg, CD45.1) and recipient (eg, CD45.2) cells would be expected for any recirculating leukocyte population.[12,15,29,30] Secondly, an intravascular anti-CD45 antibody can be administered pre-mortem to label circulating immune cells prior to organ harvest. In this case, tissue-resident cells outside of the vasculature remain unlabeled.[31] Thirdly, imaging tissue sections enables the position of immune cells relative to the vasculature to be directly defined. Together, these approaches have been used to identify tissue-specific markers expressed on organ-resident populations, for example, for tissue-resident memory T cells (Trm) these include CD69, integrin αE (CD103), and the α1 subunit of the α1β1 integrin,

CD49a.[31-34] In humans, assessing tissue residency is more challenging, but T cells isolated from non-lymphoid organs express some of these markers[35,36]; For example, CD69 is detectable on skin-resident T cells in humans.[37,38] However, there are differences in the phenotypes of Trm in murine versus human organs[38] and different organs imprint distinct tissue-specific transcriptional programmes, phenotypes, and functions on resident T cells.[35,39] To summarize, identifying and studying *bona fide* tissue-resident subsets requires careful application of the experimental systems discussed above, and is particularly challenging in humans, but is necessary to definitively delineate the organ-specific transcriptomic and epigenetic profiles of resident immune cells.

2.3 | Tissue cues, structural and immune cells in the kidney

Every organ presents a unique environment for the immune cells residing there, with specific tissue cues, shaped by the homeostatic function of the organ. In many cases, there are discrete microenvironments within an organ, due to spatial separation of different organ functions. A good example of this is found in the mammalian kidney, an organ specialized for the removal of metabolic waste and excess fluid. Anatomically, each kidney consists of an outer cortex containing glomeruli where filtrate is generated, and an inner medulla where urine is concentrated (Figure 1A). The functional subunit of the kidney is the nephron, made up of a glomerulus, proximal tubule (PT) (where filtered electrolytes are reabsorbed), loop of Henle (LOH) (that generates the intrarenal sodium gradient required for urine concentration), and collecting ducts (CD) that coalesce in the kidney pelvis.[40] Different mononuclear phagocyte (MNP) populations are differentially located in cortex and medulla.[39] Furthermore, immune cells in the cortex are exposed to a very different environment compared to medullary immune cells that experience hypersalinity and hypoxia.[41] Notably, these environmental cues can affect immune cell recruitment and function; we found that high extracellular sodium augmented the anti-bacterial function of macrophages, and increased the production of monocyte-recruiting chemokines by epithelial cells,[40] effects mediated at a molecular level by the transcription factors Nuclear Factor Of Activated T Cells 5 (NFAT5) and Hypoxia Inducible Factor 1 Subunit Alpha (HIF1α).[42] We recently applied single-cell RNA sequencing (scRNAseq) to more comprehensively profile kidney immune cells,[26] utilizing technological advances that have enabled high-throughput scRNAseq and the generation of organ atlases.[43] In normal human kidney, we identified more than 15 subsets of tissue immune cells based on their transcriptional profiles, with the immune landscape dominated by MNPs, including macrophages and DCs, T cells, and NK cells, and different populations were enriched in different regions of the kidney (Figure 1B-C). The localization pattern of the kidney immune cells was orchestrated by immune-epithelial cross-talk for regulating anti-bacterial immune responses (Figure 1D).

3 | EPIGENETIC MECHANISMS

Epigenetic mechanisms play a crucial role in cell fate specification by regulating gene expression and silencing in a context-dependent manner. Epigenetic control of gene transcription and translation does not involve changes to the DNA sequence, but rather, reversible chemical modifications of DNA or histones, or the activities of non-coding RNAs, that together enable cell- and tissue-specific, gene expression patterns that are essential for controlling normal developmental processes and maintaining tissue homeostasis. Disruption of epigenetic mechanisms can lead to organ dysfunction and disease states, including autoimmunity and cancer.

3.1 | DNA methylation

DNA methylation is one of the best-studied epigenetic modifications to date. In general, DNA methylation leads to the addition of a methyl group to the fifth carbon atom of a cytosine (5mC) followed by a guanine base (Figure 2A). As a result, the methylated CpG dinucleotides, which are frequently found in gene regulatory regions, block transcription factor binding to gene promoters, and repress target gene expression. Currently, three active DNA methyltransferases (DNMTs) are known to catalyze DNA methylation in mammals, namely DNMT1, DNMT3A, and DNMT3B.[44] Demethylation in the mammalian genome is mediated by the TET (Ten-Eleven Translocation) family of dioxygenases that oxidize 5mC to 5-hydroxymethylcytosine (5hmC), and then to 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC).[45] The "intermediate" 5hmC marks active demethylation, plays distinct epigenetic roles, and is a useful indicator of gene expression.[46,47]

DNMT1 is essential for maintaining DNA methylation at the synthesis phase of cell cycle,[48] while DNMT3A and DNMT3B are required for de novo DNA methylation. During embryogenesis, DNA is first demethylated by TET1, TET2, and TET3, resulting in a "clean slate" for de novo DNA methylation by DNMT3A and DNMT3B.[49] The activities of these two enzymes are regulated by their expression pattern and structure of distinct isoforms; DNMT3A has two isoforms DNMT3A1 and DNMT3A2.[50] Full-length DNMT3A1 expression is maintained in differentiated cells and its intact N-terminal region can interact with DNA to repress gene expression. In contrast, DNMT3A2, which lacks 223 amino acids in its N-terminal region, is predominantly expressed in embryonic stem cells (ESC). DNMT3B has more than 30 isoforms with distinct catalytic and regulatory activities. Although loss-of-function studies of DNMT3A and DNMT3B confirmed their importance in de novo methylation rather than imprinted methylation patterns,[51] double knockout mice have a gradual loss of DNA methylation over time in ESCs, suggesting that these enzymes work in concert with DNMT1 to maintain DNA methylation.[52,53] DNMT2, which is now known as tRNA aspartic acid methyltransferase 1 (TRDMT1), was found to be a tRNA methyltransferase that does not methylate DNA, despite including a similar catalytic domain to DNMT1.[54]

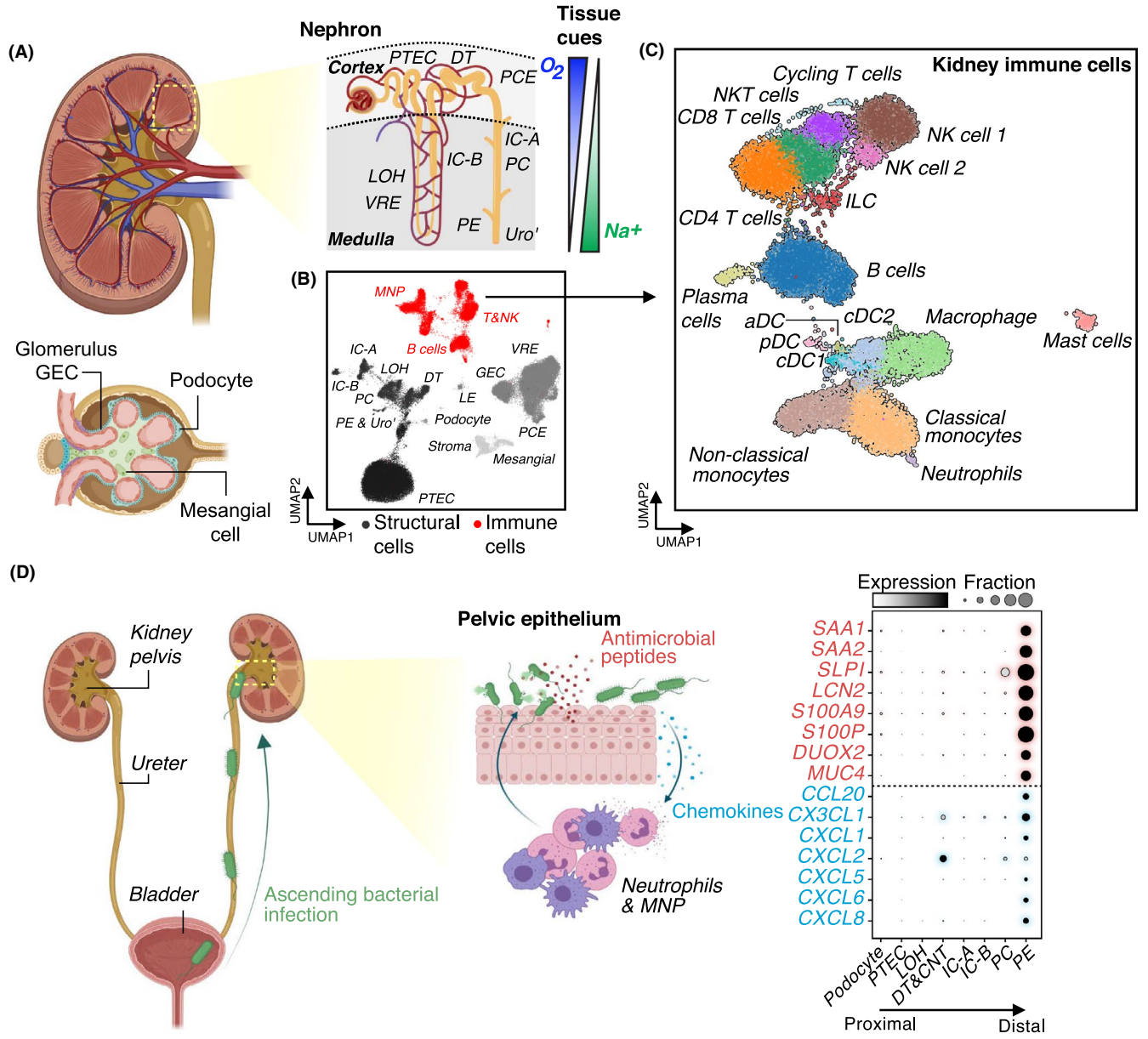

FIGURE 1 Tissue immunity in the human kidney. A, The human kidney in section; the kidney is macroscopically divided into cortex and medulla. Hundreds of thousands of nephron units are arranged over cortico-medullary depth (highlighted). Filtrate is generated in the glomerulus (lower panel) comprising podocytes, mesangial cells, and glomerular endothelial cells (GEC), before being modified by solute and metabolite resorption, excretion, and concentration along tubular nephron segments. Gradients of oxygen tension and salinity exist between the cortex and medulla as indicated. B, UMAP showing populations identified by integrated analysis of scRNAseq data (Stewart et al and Kuppe et al) (91 265 cells) (PTEC, proximal tubular epithelial cell; DT, distal tubule; LOH, loop of Henle; PC, principal cell; IC-A, intercalated cell type A; IC-B, intercalated cell type B; PE & Uro', urothelium and pelvic epithelium; LE lymphatic endothelium; GEC, glomerular endothelial cell; VRE, vasa recta endothelial cell; PCE, peritubular capillary epithelial cell; T&NK, T cells and NK cells; MNP, mononuclear phagocytes). C, The immune compartment of the human kidney as identified by integrated scRNAseq analysis (17 680 cells). Distinct populations of dendritic cells are evident (cDC1, conventional DC1; cDC2, conventional DC2; pDC, plasmacytoid DC; aDC, activated DC). Among innate lymphocytes, two subsets of NK cells (NK cell 1, NK cell 2) are evident, in addition to innate lymphoid cells (ILC). (D) Left panel: Ascending bacterial infection from the lower urinary tract first reaches the pelvic region of the kidney. Here, antimicrobial peptide production by the pelvic epithelium acts as a first line of defense, and chemokine expression orchestrates the recruitment of neutrophils and mononuclear phagocytes. Right panel: antimicrobial peptide (red) and chemokine (blue) gene expression patterns in nephron cell types ordered along the proximal-distal axis of the nephron (analysis of scRNAseq data, Stewart et al)

DNA methylation is critical for the differentiation of hematopoietic stem cells (HSCs) that give rise to lymphoid (eg, B/T lymphocytes and NK cells) and myeloid (eg, macrophages and dendritic cells) lineages. Whole DNA methylome mapping efforts have revealed reciprocal increased or decreased levels of DNA methylation during lymphoid or myeloid lineage specification, respectively.[55]

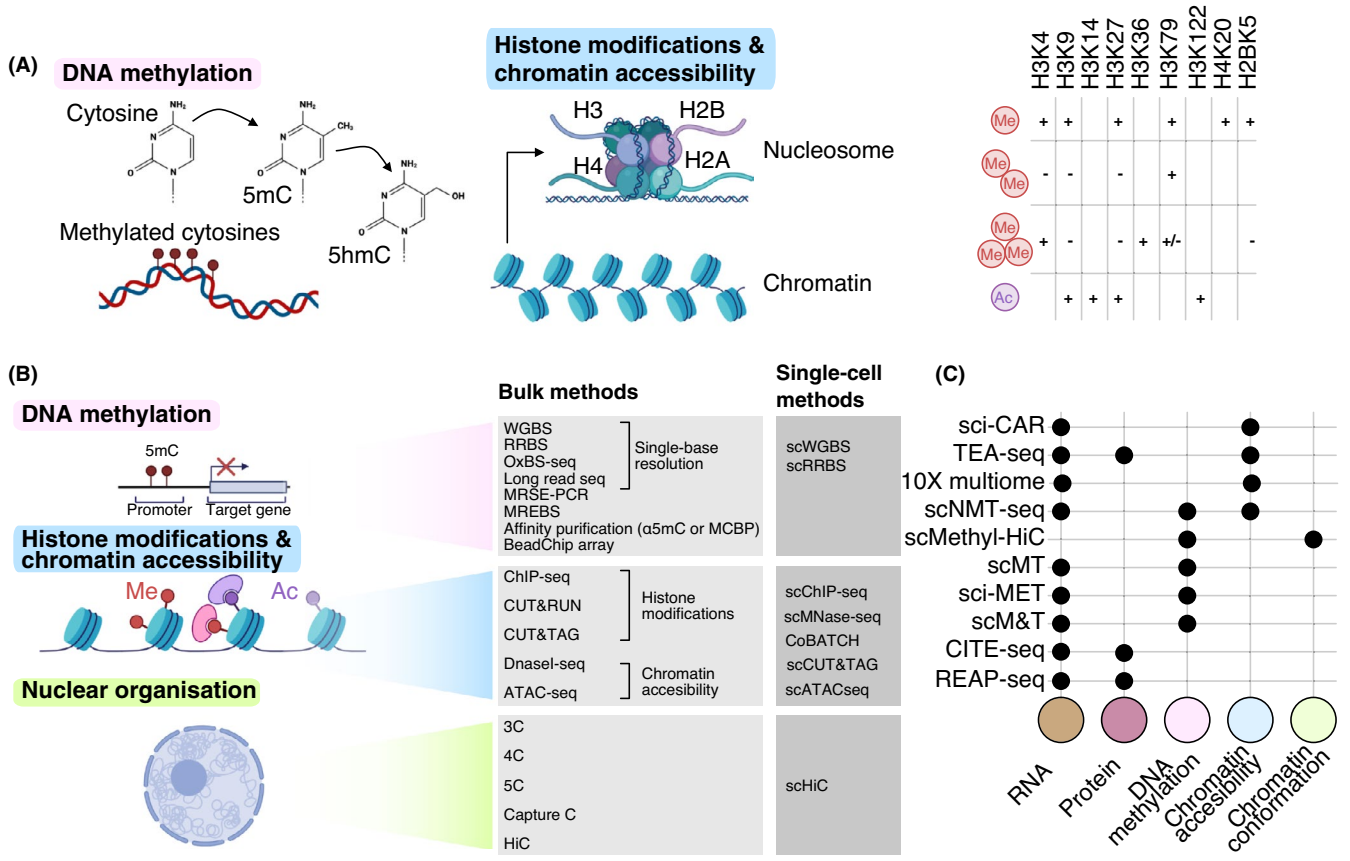

FIGURE 2 Methods to study epigenetics at bulk and single-cell resolution. A, Epigenetic modifications occur through DNA methylation (left panel) or histone modifications (right panel). Highlighted are reactions methylating cytosine pyrimidine bases to 5-Methylcytosine (5mC) and oxidation of this to 5-Hydroxymethylcytosine (5hmC). The nucleosome is composed of pairs of histone components, and commonly studied histone modifications (monomethylation, dimethylation, trimethylation, and acetylation) and their effect on chromatin structure and transcription are highlighted (+, activation; -, repression). B, Outline of methods to study DNA methylation, histone modifications, chromatin accessibility, and nuclear organization for both bulk sample and single-cell applications. C, Illustration of multi-omic profiling methods, highlighting the molecular layer targeted by each assay

The generation of functionally specialized, differentiated immune cell subsets is regulated by DNA methylation, for example, regulatory T cell (Treg) differentiation is coordinated by DNMT1,[56] T helper (Th)1/2/17 polarization by TET2 and DNMT3A,[57,58] and memory T cell activation by demethylation of interferon gamma (IFNG).[59] These loss-of-function studies have not only revealed the role of these enzymes in methylation or demethylation of specific CpG sites, but also show their influence in controlling the activity of transcription factors that may bind to enhancers in these cell types.

3.2 | Histone modifications

DNA is highly compacted into nucleosomes, obstructing transcription, but post-translational modification of nucleosome core histones at their N-terminus may alter the accessibility of DNA to the transcriptional machinery, resulting in the activation or repression of gene expression[60] (Figure 2A). Nucleosomes comprise 145-147 base pairs (bp) of DNA wrapped twice around eight core histone proteins (H), including two copies of H2A, H2B, H3, and H4, and adjacent nucleosomes are joined by 10-70bp of linker DNA to form nuclear chromatin. The N-terminal regions of histones contain modifiable amino acids at their surface, including lysine, arginine, serine, threonine, and tyrosine. Chromatin compaction and accessibility are altered by chemical modification of these amino acids, specifically acetylation, methylation, and phosphorylation, changing the transcription of certain genes.

Histone acetylation usually results in higher gene expression and is achieved by the regulated activity of two groups of enzymes with opposite effects, namely histone acetyltransferases (HATs) and histone deacetylases (HDACs). HATs were first identified by Allfrey et al[61] who found that these enzymes can neutralize lysine's positive charge in the tail regions of histones by transferring an acetyl group from acetyl-CoA to the target lysine residues, weakening the interaction between histones and DNA. As the modified chromatin becomes less compact, the transcriptional machinery can gain access to target genes promoting transcription. HATs fall into two classes: type-A and type-B. While type-B HATs, such as HAT1, acetylate newly synthesized histones H3 and H4 at their tail regions in the cytoplasm,[62] type-A HATs such as MYST, Sas (Something about

silencing)2, Sas3 (previously yeast Ybf2), TIP60 (Tat-interacting protein 60 kDa)), and Cyclic adenosine monophosphate (cAMP) Response Element-Binding Protein (CREB)-binding protein (CBP)/p300 families, acetylate nucleosomal histones in the context of chromatin.[63] On the other hand, HDACs oppose the actions of HATs, restoring lysine's positive charge that is essential for the stability of the chromatin architecture,[63,64] leading to closed chromatin and suppressing gene expression.

Like histone acetylation, phosphorylation of histones at serines, threonines, and tyrosines is also dynamic, and regulated by counteracting kinases and phosphatases. Kinases transfer a phosphate group from ATP to the target residues, adding negative charge to the N-terminal regions of histones (for example, mitogen-activated protein kinase 1 (MAPK1)[65]) or to the core region (for example, Janus kinase 2 (JAK2)[65,66]), creating sites for DNA exit from the nucleosome. Histone phosphorylation works in partnership with other modifications, for example, phosphorylation of the serine in histone H3 (H3S10ph) leads to further acetylation at H3K9ac,[67] H3K14ac,[68] and H4K16ac.[69]

Unlike acetylation or phosphorylation that alters gene expression patterns by changing the charge of histones, the addition of one or more methyl groups at lysines and arginines of histones enables DNA to uncoil from nucleosomes. Histone methylation can lead to transcriptional repression or activation, depending on the amino acid targeted and the number of methyl groups added. These factors are determined by critical residues in the catalytic domains of histone lysine methyltransferases (HKMTs), including DIM5 (defective in methylation 5) and SET (Suppressor of position-effect variegation (Su(var))3-9, Enhancer-of-zeste, Trithorax)7/9. X-ray crystallography[70,71] and site-directed mutagenesis[72] have shown that Tyrosine281 in DIM5 or Phenylalanine305 in SET7/9 is responsible for transferring one or three methyl groups to target histone lysines, respectively. Generally, methylation of Lysine9 (H3K9me2, H3K9me3) and Lysine27 (H3K27me2) in H3 leads to repression of transcription, and these modifications are enriched in developmentally silenced loci or heterochromatin domains.[73] In contrast, methylation of Lysine4 (H3K4me1, H3K4me2, H3K4me3), Lysine36 (H3K36me3), and Lysine79 (H3K79me1) in H3 results in active transcription, and are present at the 5' untranslated region of target genes (H3K4), or in gene bodies (H3K36, H3K79).[73,74]

3.3 | Non-coding RNAs

Non-coding RNAs comprise the majority of RNAs, do not encode functional proteins, and play a pivotal role in regulating gene expression at the post-transcriptional level. These non-coding RNAs include micro-RNAs (miRNAs), Piwi-interacting RNAs (piRNAs), small interfering RNAs (siRNAs), and long non-coding RNAs (lncRNAs). miRNAs and siRNAs contain 19 ~ 24 nucleotides, can both be generated through cleavage by the RNase III enzyme Dicer, and induce RNA silencing by forming a RNA-induced silencing complex (RISC) with their target mRNAs. miRNAs are single-stranded RNAs that contain incomplete hairpin structures and achieve gene silencing by targeting untranslated regions of enzymes critical for chromatin remodeling, such as HDAC4 (miR-140,[75] POLR3D by miR-320[76]), and DNMT3A and DNMT3B by miR-29 family.[76,77] In addition to their silencing effects, recent studies have shown that miRNAs such as miR-373 may induce RNA activation through binding to the promoter regions of target genes, for example, E-cadherin and CSDC2.[78] piRNAs are transcripts with 26 ~ 31 nucleotides that form RNA-protein complexes by interacting with Piwi domain-containing Argonaute proteins to achieve target gene silencing,[79] particularly of transposons.[80] The biogenesis of piRNAs is not yet clear; however, it is thought that they could be derived from long, single-stranded precursor molecules,[81] catalyzed by two piwi proteins Aubergine (Aub) and Argonaute-3 (Ago3). This process, also known as the piRNA Ping-Pong pathway, appears to trigger the degradation of transposons.[82] lncRNAs transcripts exceed 200 nucleotides in length, for example, X-inactive specific transcript (XIST), a 17kb lncRNA best known for its role in X chromosome inactivation. XIST physically binds to its target X chromosome in cis, recruiting the Polycomb complex 2 (PRC2).[82] As a result, H3K27 trimethylation is induced to repress gene expression.

4 | TECHNOLOGIES: PROFILING EPIGENETIC MECHANISMS—MOVING FROM TISSUES TO SINGLE CELLS

4.1 | DNA methylation profiling

Genome-wide assays of DNA methylation (5mC) can be performed using methods which capture the entire genome at single-base resolution, or methods that target specific modifications and regions of the genome to build lower resolution maps of methylation (Figure 2B). The current gold standard is whole-genome bisulfite sequencing (WGBS). A high concentration of sodium bisulfite at pH 5.0 results in deamination of cytosine to uracil, while 5mC is protected from the deamination reaction.[83] Consequently in sequencing data, 5mC are read as cytosine bases; however, the deaminated bases are sequenced as thymines. In the original methodology, Sanger sequencing was used to assess CpG methylation.[83,84] The current standard approach is to prepare libraries for next-generation sequencing (NGS) to generate genome-scale maps of DNA methylation.[85]

WGBS cannot differentiate 5mC and 5hmC—both are read as cytosines in sequencing. A modification to bisulfite sequencing, oxidative bisulfite sequencing (OxBS-seq), converts 5hmC to 5fC, and subsequent bisulfite treatment converts 5fC to uracil, leaving 5mC unconverted. Comparisons between oxBS-seq and conventional bisulfite sequencing allow for identification of 5hmC modified regions.[86,87] An alternative method for 5hmC profiling utilizes differential TET enzyme-mediated oxidation; in this assay, 5hmC is converted to β-glucosyl-5-hydroxymethylcytosine (g5hmC) by β-glucosyltransferase, and is protected from oxidation by TET. Consequently, 5hmC is sequenced as cytosine after bisulfite

conversion, whereas 5mC which becomes oxidized to 5caC is sequenced as thymine.[88,89]

Protocols for single-cell bisulfite sequencing (scBS-seq) have been developed, consisting of single-cell isolation into plate wells, prior to bisulfite conversion and library construction.[90] As a proof of principle, these methods have been used to interrogate DNA methylation patterns in ESC.[91,92]

WGBS remains prohibitively costly for high-throughput experiments, principally because very large genomic regions which are CpG depleted consume sequencing reads. A complementary approach—reduced representation bisulfite sequencing (RRBS)—employs DNA digestion with methylation-insensitive restriction enzymes to enrich regions of the genome enriched for CpG, prior to bisulfite conversion, PCR amplification, and sequencing.[93] This method covers only around 10%-15% of the genome and provides a biased view of the genome as a result of cleavage at restriction enzyme-sensitive sites. While RRBS offers very limited coverage of non-CpG island regions, it does offer single-base resolution data on areas dense in CpG methylation. RRBS has also been adapted to study DNA methylation patterns in single cells. Single-cell reduced representation bisulfite sequencing (scRRBS) has been used to decipher heterogeneity among ESC.[94-96]

More recently, methods to simultaneously assay single-cell methylome and transcriptome have been developed, relying on physical separation of RNA and genomic DNA. These include Switching Mechanism At the end of the 5'-end of the RNA Transcript (Smart)-RRBS and scMT-seq which combine the Smart-seq2 whole transcriptome method[97] and RRBS,[98,99] scM&T-seq which adapts the existing G&T-seq approach[100] with a bisulfite conversion step for genomic DNA.[100,101] Data from single-cell methylation profiling approaches suffer from the limited throughput and high cost per-cell. In an attempt to increase throughput, Mulqueen et al[102] leveraged combinatorial indexing in a method termed sci-MET. Using this approach, they use WGBS to profile cell lines and murine primary cortical nuclei, overall generating 3282 scBS libraries. These data illustrated the potential of this sc-WGBS to distinguish cell types in primary tissue in a manner amenable to scaling.

Methylation-specific restriction enzyme (MRSE)-based approaches take advantage of restriction endonucleases which are sensitive to base methylation status. Here, libraries can be generated after cleavage of DNA by restriction enzymes unable to cleave methylated-cytosine bases. Methylated regions will remain intact after DNA cleavage. These fragments are amplified by PCR and sequenced. In contrast to bisulfite sequencing, this method does not offer single-base resolution.[103]

A hybrid approach—methylation-sensitive restriction enzyme bisulfite sequencing (MREBS) builds on the strengths of MRSE and RRBS, and extends the coverage of RRBS to a larger fraction of the genome.[104]

Using an affinity purification approach, 5mC specific antibodies[105] or methyl-CpG binding protein[106] can be used to enrich regions of the genome which are highly methylated. After immunoprecipitation, fragments enriched for 5mC can be assayed by array hybridization or next-generation sequencing. This method is highly economical but biases toward hypermethylated CpG rich regions of the genome.[107]

A further method providing a low-cost and high-throughput view of the methylome uses bisulfite conversion of genomic DNA followed by PCR amplification and hybridization to a microarray. This method—the Illumina HMEPIC BeadChips generate data on 850 000 methylation sites across the genome, and build on the 450 000 site HMK450 chip predecessor.[108] Although this does not provide single-base resolution, it does offer coverage of the 95% of CpG islands and is well suited to high-throughput approaches assaying methylation variation in population studies.

Long-read methods including nanopore sequencing, and SMRT sequencing have also been used to assay methylation status genome-wide without requiring bisulfite conversion via picoampere signal intensities corresponding to modified bases (Nanopore),[109] or variation in polymerase kinetic activity (SMRT sequencing).[110] Although these methods generate data with a higher error rate and modest throughput, they are able to provide much longer reads allowing more efficient interrogation of methylome haplotypes[111] and delineation of methylation status at repetitive elements and structural variants.[112]

4.2 | Histone modifications

Genome-wide profiles of chromatin modifications can be routinely assayed using chromatin immunoprecipitation followed by sequencing (ChIP-seq) (Figure 2B). This method utilizes protein affinity purification using an antibody specific for a chromatin post-translational modification or another DNA-binding protein such as a transcription factor. Following crosslinking of DNA-protein complexes, fragmentation, and exonuclease treatment, DNA-protein complexes are immunoprecipitated, and enriched DNA fragments are sequenced using NGS.[113-116] This method has been extremely widely used to profile genomic regions associated with transcription factors, and a broad range of histone acetylation and methylation states.

Attempts to generate ChIP-seq data at single-cell resolution have used droplet-encapsulation microfluidics to perform massively parallel DNA barcoding followed by NGS. Rotem et al[117] profiled H3K4me3 and H3K4me2 marks in mouse ESC, embryonic fibroblasts (EF), and hematopoietic progenitors. The method ligates DNA barcodes to chromatin-associated DNA fragments generated after MNase treatment, providing an index link to the cell of origin. The method then proceeds to immunoprecipitation on a pooled sample, and sequencing of enriched DNA fragments. While these data are extremely sparse, the histone modification profiles generated readily distinguished ESC and EF, and revealed heterogeneity among the gene regulatory programmes of ESC. Building on this droplet microfluidics-based approach, Grosselin et al[118] profiled H3K4me3 and H3K27me3 marks firstly in human B- and T lymphocytes, before turning to murine stromal cells. In a patient-derived xenograft model of triple-negative breast cancer,

they were able to detect rare untreated cells bearing the same H3K27me3 repressive pattern as drug-resistant cancer cells. They speculated that this may represent epigenetic priming of cancer cells toward treatment resistance, a feature not readily identifiable by gene expression profiling.

Single-cell or low-input ChIP-seq is technically very challenging, largely as a result of non-specific immunoprecipitation. An alternative approach termed CoBATCH dispenses with chromatin immunoprecipitation, and instead utilizes a protein A-Tn5 transposase fusion protein to perform in situ targeted tagmentation at sites bound by specific antibodies, before a round of combinatorial indexing. Using this method, Wang et al[119] profiled 2758 endothelial cells sorted from mouse embryonic organs for the H3K27ac mark to define regions with active enhancers, identifying numerous organ-specific H3K27ac profiles at transcription factor enhancers, for example, *Hoxa11* in the kidney.

In 2017, Skene and Henikoff developed Cleavage Under Targets & Release Using Nuclease (CUT&RUN) for mapping interactions between DNA and proteins. This method offers technical advantages over ChIP-seq, such as avoidance of false-positive site identification as a result of cross-linking. In common with ChIP-seq, this method uses transcription factor-specific antibodies. Nuclei are immobilized using concanavalin-A coated magnetic beads. After the addition of a protein A-MNase fusion protein and free Ca^{++}, DNA cleavage occurs at antibody-associated sites, releasing transcription factor-associated fragments which diffuse out of the nuclear membrane and into the supernatant which are collected for library preparation and NGS. The method offers lower background signal, and reduced sequencing requirements and hence represents a cost-effective alternative to ChIP-seq.[120] The same group followed the CUT&RUN method with Cleavage Under Targets and Tagmentation (CUT&Tag). Similarly to CoBATCH, this method uses a protein A-Tn5 transposase fusion protein and generates fragment libraries at antibody-targeted sites, allowing the profiling of histone modifications and transcription factor associated DNA in low-input material and single cells.[121] By virtue of the use of a Tn5 transposase, this method generates a low-level Assay for Transposase-Accessible Chromatin using sequencing (ATACseq) signal in addition to a strong protein-enriched signal, therefore offering the potential for delineation of joint chromatin accessibility and binding protein associated profiles. Future developments are likely to extend this method to probe multiple histone modifications and transcription factors via adapter barcoding. The utility of a protein A-Tn5 transposase fusion protein in generating single-cell histone modification maps has been replicated by Carter et al[122] Although a single-cell method employing targeting of MNase to specific antibody bound sites successfully profiled H3K4me3 on 242 cells, this approach lacks scalability.[123] The CUT&Tag approach has since been adapted to commercial, high-throughput droplet microfluidics single-cell ATACseq protocols (scATACseq), generating datasets on histone modification patterns in the murine central nervous system.[124]

4.3 | Chromatin accessibility

Mapping regions of open chromatin across the genome were initially achieved through identifying sites sensitive to deoxyribonuclease I (DNase I)[125,126] (Figure 2B), a nuclease that preferentially cleaves DNA at phosphodiester linkages adjacent to pyrimidine nucleotides. With the advent of high-throughput sequencing, investigators were able to map DNase I cleavage regions throughout the genome, termed DNase-sEquations [127,128] DNase sensitivity maps have been instrumental in efforts to produce comprehensive inventories of DNA elements, exemplified by the ENCODE project.[129-131]

Genome-wide profiling of chromatin accessibility has been enabled through the use of Tn5 transposase loaded with sequencing adapters. The enzyme inserts adapters at regions of open chromatin and generates DNA fragments for downstream NGS profiling.[132] Recent improvements to the ATACseq protocol, namely Omni-ATAC and Fast-ATAC, have been demonstrated to have substantially reduce background noise in diverse cell lines and tissue types.[133,134] Omni-ATAC also worked on frozen sample blocks, which were historically difficult to assay.[133] Fast-ATAC is an optimized ATACseq protocol for blood cells, which produced high-quality data with reduced noise.[134] ATACseq has been adapted to a high-throughput single-cell method, initially as a nano-well based method,[135] before its evolution to a massively parallel droplet-microfluidics implementation capable of profiling hundreds of thousands of cells simultaneously.[136] Initial work using this method has illustrated its utility in studying dynamic developmental processes, chiefly epigenomic differentiation trajectories during haematopoeisis.[135,136] Integrating genomic variants associated with blood cell traits, with regions of accessible chromatin during hematopoiesis, has allowed investigators to probe genome regulation across blood cell lineages.[137]

scATACseq has also found utility in distinguishing clonal relationships between cells via mitochondrial mutation tracing. As the mitochondrial genome is non-chromatinized, scATACseq (but not snATACseq) generates abundant reads at high coverage across the mitochondrial genome. This provides a valuable source of information on somatic mitochondrial mutation patterns. Tracing clones and differentiation trajectories across an in vitro model of hematopoiesis, Lareau et al[138] were able to identify clones with distinct lineage biases and applied their clonal tracing method to in vivo hematopoeisis.

Taking advantage of combinatorial indexing, Cao et al[139] report a method they term sci-ATACseq3, which permits generation of massive-scale scATACseq data. They used this to generate a pan-fetal atlas of human development across multiple time points, reporting on around 800 000 cells. Within this data set, the authors identified macrophages with distinct chromatin accessibility patterns according to the tissue of origin, and a subset of highly phagocytic macrophages restricted to the spleen and liver—likely representing nascent iron recycling macrophages.

4.4 | Chromatin conformation

The three-dimensional organization of the genome within the nucleus can bring distant domains close together and can act as an important set of regulatory mechanisms.[140] These structures include the 30 nm fiber, chromatin loops, and interchromosomal interactions. The systematic study of genome topology has been enabled by Chromosome Conformation Capture (3C) (Figure 2B). In this method, interactions between distant loci are captured by formaldehyde crosslinking of chromatin. Thereafter DNA is digested by restriction enzymes generating crosslinked fragments, which are then re-ligated forming chimeric DNA molecules for subsequent PCR of target loci.[141] The development of this technology has spawned two decades of adapted techniques using sequencing to capture chromatin organization, including 4C, 5C and, capture-C,[142] and HiC.[143,144] Similarly to the original 3C method, HiC generates chimeric DNA fragments following formaldehyde crosslinking. These are subjected to paired end sequencing, before mapping to the genome, followed by computation identification of higher-order interactions such as chromatin loops and Topologically Associating Domains (TADs). HiC is therefore able to uncover the contact probabilities of DNA across the entire genome. This method has been applied to the study of immunity, investigating dynamic remodeling of chromatin conformation during T cell development and activation.[145-147]

HiC has been extended to a single-cell application, allowing the structural modeling of chromosomes and probing of the intercellular variability in structures. Nagano et al[148] determined that across a homogeneous population of murine $CD4^+$ Th1 single cells, active genes localize to the interfaces or boundaries between territories, and that higher-order chromatin structures are highly variable between cells, whereas domain organization is well preserved at the megabase scale. Scaling up the single-cell HiC approach, Ramani et al[149] applied combinatorial indexing, to generate scHiC data on 10, 696 cells, and distinguish cells on the basis of cell-cycle state and karyotype.

4.5 | Multimodal single-cell technologies

Development of highly sensitive methods for chromatin accessibility measurements and gene expression measurements have largely progressed in parallel—recently methods for single-cell multi-omic profiling have gained traction.[150] Using a pooled barcode methodology, Cao et al[151] reported joint chromatin accessibility and gene expression profiling on thousands of murine kidney cells. In the same year, a method for single-cell joint profiling of chromatin accessibility, DNA methylation, and transcription was reported.[152] A method for joint DNA methylation and chromatin conformation has been developed, combining HiC and WGBS (scMethyl-HiC), and Li et al[153] demonstrated this method's capacity to delineate heterogeneity among murine ESCs. More recently, large-scale multi-omic profiling spanning chromatin accessibility, gene expression, and protein expression has been performed, generating multimodal data (Figure 2C) on human hematopoiesis and peripheral blood, paving the way for future studies interrogating molecular regulatory programmes in more challenging samples such as tissue-resident immune cells and solid organ parenchymal cells.[154,155]

5 | EPIGENETIC CONTROL OF TISSUE IMMUNE CELLS

Given the importance of epigenetics in controlling cell fate specification and subsequent function, delineating tissue-specific epigenetic controllers in resident immune cells has gained increasing interest. The development of robust assays (discussed in Section 4) has enabled key mechanisms controlling context-specific gene expression to be profiled for the major tissue-resident immune cell populations, which will be discussed in turn.

5.1 | Macrophages

Macrophages are critical tissue immune sentinels and regulate many aspects of the immune response to ensure the maintenance of organ homeostasis. Macrophages are present in virtually all tissues, including the kidneys, and their ontogeny can be traced to two distinct developmental origins. They are either embryonically seeded from early YS or fetal liver progenitors or from infiltrating monocytes and monocyte precursors, which arise from bone marrow-derived hematopoietic stem cell (HSC) progenitors[7,156] (Figure 3). Elegant fate mapping studies in mice tagged with a C-X3-C Motif Chemokine receptor 1 (*Cx3cr1*)-green fluorescent protein reporter system showed that YS cells and mature macrophages from YS-lineage could be identified by higher expression of F4/80 ($F4/80^{high}$) and intermediate expression of CD11b ($CD11b^{int}$) while HSC-derived macrophages and monocytes were low in F4/80 ($F4/80^{low}$) but high in CD11b ($CD11b^{high}$) expression even in adult tissues, including the kidney.[7] PU.1 is a key transcription factor for myelopoiesis and antagonistically interacts GATA1 to regulate activation/repression of transcription by recruiting transcriptional machinery leading to methylation of histone H3K9 for repressing GATA1 activity, or together with its coactivator, cJun, promotes histone H3K9 acetylation for active transcription of target genes[157] (Figure 3). While knocking out PU.1 depleted all macrophages non-discriminately, which was necessary for macrophage but not HSC development,[158-160] knocking out Myb showed that only $CD45^+F4/80^{low}CD11b^{high}$ cells were absent in all tissues examined, with $CD45^+F4/80^{high}CD11b^{int}$ cells constituting the majority macrophage population,[7] consistent with the requirement of Myb for development of HSCs but not YS cells.[161-163] Even though both ontological sources of macrophages homeostatically replenish the core network of tissue-resident macrophages throughout life, the former is thought to possess self-renewing capacity within the tissue,[164] and were transcriptionally distinct to their HSC-derived counterparts.[7] Although fate mapping studies have been limited to mouse, these ontological relationships are thought to hold true in human tissue macrophages, supported by recent single-cell

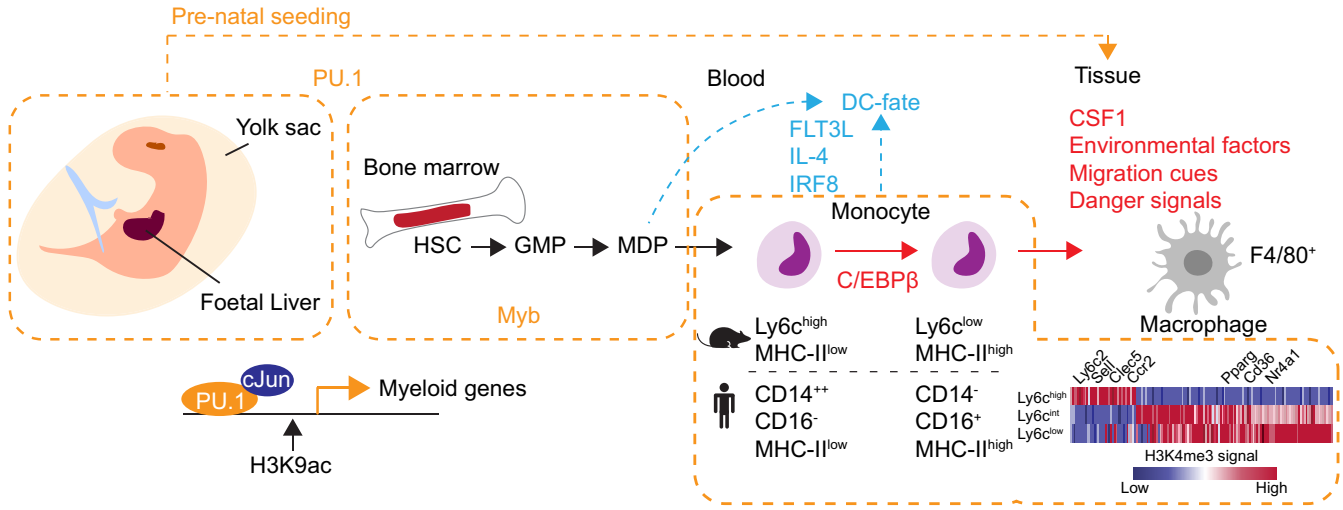

FIGURE 3 Epigenetic control of macrophage development. The expression of key transcription factors, appropriate cytokine signaling, local environmental factors and danger signals govern monocyte and macrophage development. Tissue-resident macrophages can arise from either pre-natal seeding of YS progenitors or development from monocytes from the bone marrow. PU.1 is a master regulator of myelopoiesis and interacts with co-activate cJun, leading to acetylation of H3K9 in promoter regions of target genes. C/EBPβ regulates the development of Ly6c^{hi} to Ly6c^{lo} monocytes. Expression of C/EBPβ is correlated with gain/loss of relevant histone modification marks relevant for monocyte/macrophage gene programs. In the tissue, macrophages then receive further signals to support their differentiation

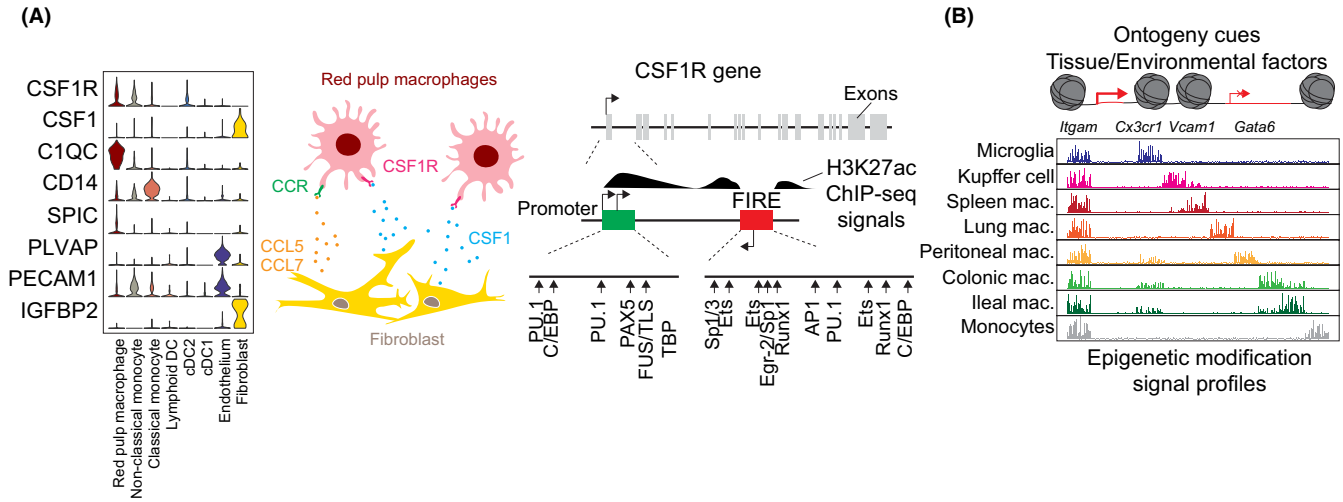

FIGURE 4 Transcriptional control of survival and tissue specification in macrophages. A, Supporting cells in tissues can express cytokines to support a survival niche for macrophages in tissues, for example, fibroblasts in the spleen express CSF1, maintaining a local network of red pulp macrophages. Regulation of the CSF1R gene is controlled by a super-enhancer element, FIRE, which is flanked by H3K27ac histone marks. Adapted from Rojo et al.[181] PU.1 can also bind to FIRE. B, The epigenetic landscape is molded by both ontogeny cues and organotypic factors, resulting in accumulation of epigenetic modifications that enable or inhibit differential expression of genes that result in distinct fate specifications for various monocyte/macrophage cell types in different organs. Adapted from Lavin et al[183]

RNA-seq studies of macrophages in fetal and adult organs,[156,165] as well as in humanized mice.[166] Indeed, in human adult kidneys, we defined transcriptomically distinct clusters of mononuclear phagocytes (MNPs) as tissue-resident macrophages or monocyte-derived macrophages based on transcriptional similarity to human fetal kidney macrophages, as well as fate-mapped YS macrophages in mouse.[26]

In mice, transitioning of short-lived Ly6C^{high}MHC-II^{low} (major histocompatibility complex 2) monocytes to mature Ly6C^{low}MHC-II^{high} monocytes was found to be dependent on CCAAT-enhancer-binding protein beta (C/EBPβ), which binds to the promoter of *Nr4a1* (also known as *Nurr77*) and induced its expression,[169] maintaining a survival gene expression program in maturing monocytes.[167,168] This correlated with the loss/gain of H3K4me histone marks on promoters for relevant genes such as *Ly6c2*, *Sell*, *Clec5*, *Pparg*, *Cd36*, *Ccr2*, and *Nr4a1*[169] (Figure 3). Chromatin accessibility was also observed to increase in regions

relevant to the expression of classical monocyte genes, for example, *Cx3cr1*, *Nr4a1*, and *Itgax*.[169]

In addition, differentiation from monocytes to macrophages requires signaling from the cytokine colony-stimulating factor 1 (CSF1) receptor (CSF1R).[170] Genetic deletion or antibody-mediated depletion of CSF1 leads to macrophage deficiency in adult tissues.[171,172] Cells that produce CSF1 provide a niche for macrophage homeostasis; for instance, Wilms' Tumour 1 (WT1)-expressing reticular fibroblasts in stromal regions of the spleen are local producers of CSF1, maintaining the red pulp macrophage network, as well as C-C Motif Chemokine Ligand 2 (CCL2) and CCL7, for recruitment of circulating monocytes[173] (Figure 4A). Regulation of CSF1R expression is in part controlled by the presence of *fms*-intronic regulatory element (FIRE), a highly conserved super-enhancer element flanked by H3K27ac marks in mouse macrophages.[174-177] Furthermore, the PU.1 transcription factor is also found to bind FIRE[178-181] (Figure 4A). Targeted deletion of FIRE using CRISPR/Cas9 in mice led to selective depletion of brain microglia and macrophages from embryo, heart, skin, peritoneum, and the kidney but retention of normal proportions of osteoclasts, liver macrophages (Kupffer cells; KCs), lung alveolar macrophages, splenic marginal zone macrophages, lung alveolar macrophages, and other lung interstitial myeloid populations and intestinal macrophages.[182] While multiple H3K4me1 (primed enhancers) marks were found within the *Csf1r* locus of various macrophage populations, ATACseq profiles for FIRE displayed high heterogeneity in different types of macrophages, explaining some of the selective depletion effects observed in the FIRE-deficient mice; for example, ATACseq peaks for FIRE in lung and intestinal macrophages were much lower than that observed in brain microglia, suggesting that chromatin accessibility for FIRE is relatively greater in microglia.[182,183]

The transition of human monocytes to macrophages is also dependent on the regulation of the epigenome. For instance, the BLUEPRINT consortium performed genome-wide epigenome profiling (via DNase I-seq, ChIP-seq, and RNA-seq) on monocytes extracted from human peripheral blood, as well as macrophages, generated ex vivo from monocytes, cultured in the presence or absence of various stimuli.[184] They found that ~8000 regions were hypersensitive to DNase I cleavage and marked by differential histone modification marks in the monocytes compared with macrophages.[184] Differential expression of transcription factors, paired with DNA-binding motif sequence scanning, highlighted the differential enrichment of TF-binding motifs within DNaseI hypersensitive sites after different macrophage stimulations, pointing toward key epigenome changes related to macrophage tolerization toward lipopolysaccharide (LPS) stimulation versus a "trained" response[184] (see section on trained immunity). Similarly, there was localized gain or loss of DNA methylation across genome-wide CpG sites along the spectrum of human monocyte to macrophage development.[185]

Beyond the developmental and ontological cues that may dictate a macrophage's cell fate and identity, the local tissue environment plays important roles in shaping macrophage differentiation. For instance, the acquisition of a macrophage program was shown to occur in tandem with organogenesis where dynamic spatial and temporal expression of key tissue-specific transcriptional regulators defined the diversity and heterogeneity of macrophages in developing mice.[5] As such, the transcriptional regulatory landscape of tissue-resident macrophages is a balance between nurture (environment) and nature (ontological lineage). Combining RNA sequencing, genome-wide ChIP-seq and ATACseq on macrophages, monocytes, and granulocytes showed broad cell type-specific histone modifications on specific enhancer elements unique to each group, for example, H3K4me3 marks were present on the promoter of *Mertk* in macrophages but not monocytes or neutrophils.[183] Furthermore, the enhancer landscape in tissue-resident macrophages was also distinct depending on the tissue/environment, as well as ontogeny[183] (Figure 4B). In a separate example, the epigenetic landscape governing KC identity and diversity in the liver is determined by spatially distinct microanatomical niches in the liver sinusoids.[186] This was associated with similar patterns of chromatin accessibility between KC and non-alcoholic steatohepatitis (NASH)-associated Tim4$^-$ KC-like recruited macrophages, which occupy similar spatial locations, compared to Ly6c2 expressing monocyte-related macrophages.[186]

Tissue macrophages are also regulated by distinct migration cues instructed by the environment. For instance, in the kidneys, we previously showed that high interstitial sodium in the medullary/pelvic zones induced CCL2 and C-X3-C Motif Chemokine Ligand 1 (CX3CL1) chemokine production by medullary tubular epithelial cells, which in turn recruits CCR2/CX3CR1-expressing CD14$^+$ monocytes[9] (Figure 5). Perturbation to the renal sodium gradient, for example, in patients with diabetes insipidus, a condition where urine concentration is impaired[187] due to reduced secretion/action of the antidiuretic hormone, vasopressin, there was reduced recruitment of monocytes to the medulla.[9] In a reciprocation fashion, in the Dahl salt-sensitive rat model, a pre-clinical model for salt-dependent hypertension and chronic kidney disease,[188] increased infiltration of macrophages, T cells, and B cells was observed. Abnormalities of tissue immune cells have also been reported with a high-salt diet,[189] including an increased ratio of CD86$^+$ to CD163$^+$ macrophages in the kidney, interpreted as an increased M1:M2 ratio.[190] Overall, salt-related effects were shown in multiple studies to be mediated by NFAT5, a key transcription factor that senses hypertonicity and regulates gene expression to enable cellular adaptation to hyperosmotic stress[191,192]; manipulation of NFAT5 expression via siRNA-knockdown in HEK 293T cells or in *Nfat5*-deficient mice (*Nfat5$^{fl/fl}$-Ert2-Cre*) resulted in decreased chemokine production at the mRNA and protein levels.[9] Furthermore, the hypersaline environment of the kidney medulla was crucial in promoting NFAT5-dependent anti-bacterial functions in mice and human kidney MNPs.[9] NFAT5-dependent activation of macrophages in a high salt environment was also observed in skin, with enhanced activation following LPS stimulation in high-salt conditions, as well as increased tumor necrosis factor alpha (TNF) and nitric oxide secretion in a model of skin parasite (*Leishmania major*) infection.[193] Specifically, LPS-related signaling pathways augmented the phosphorylation of p38/mitogen-activated protein kinase (MAPK) in high salt conditions, increasing TNF and nitric oxide.[194]

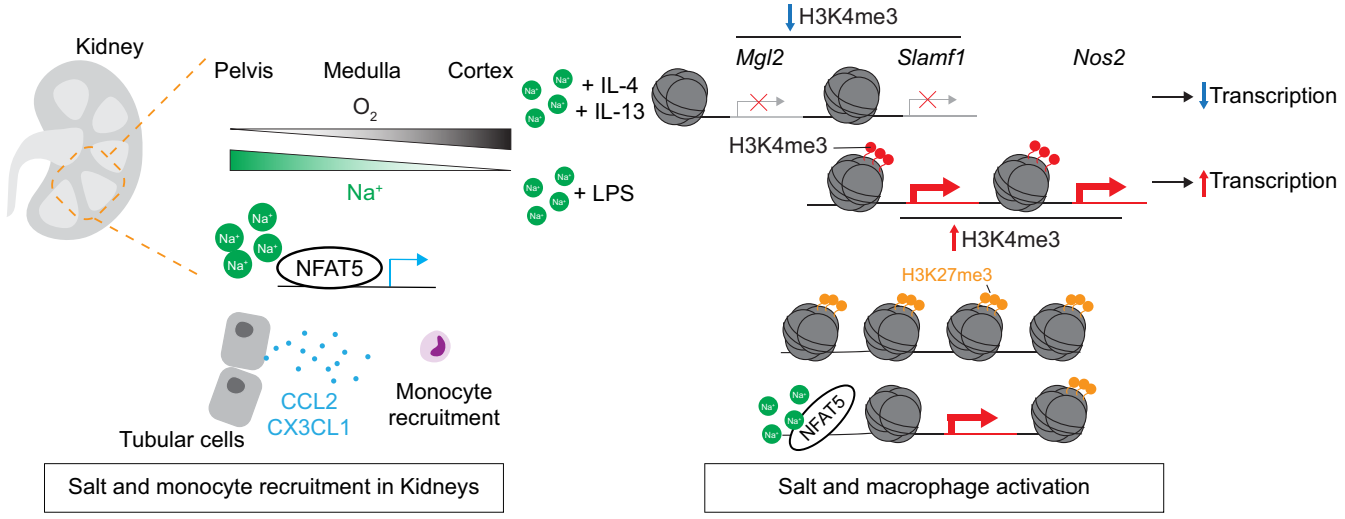

FIGURE 5 Salt-dependent NFAT5 regulation of monocyte recruitment and macrophage polarization and function. Environmental gradients in the kidney, such as salt and hypoxia regulate macrophage/monocyte recruitment signals. Salt is sensed by NFAT5, which regulates the expression of key chemokines for monocyte recruitment. Treated macrophages with IL-4 and IL-13 in a high salt concentration led to reduced deposition of H3K4me3 marks around Mgl2 and Slamf1 suggesting the M2 polarization is inhibited by high salt. LPS treatment does not promote M1 polarization, but furthers the M2 effects, shown by increased H3K4me3 marks. NFAT5 expression in macrophages is also correlated with the loss of repressive H3K27 methylation, promoting downstream immune gene expression

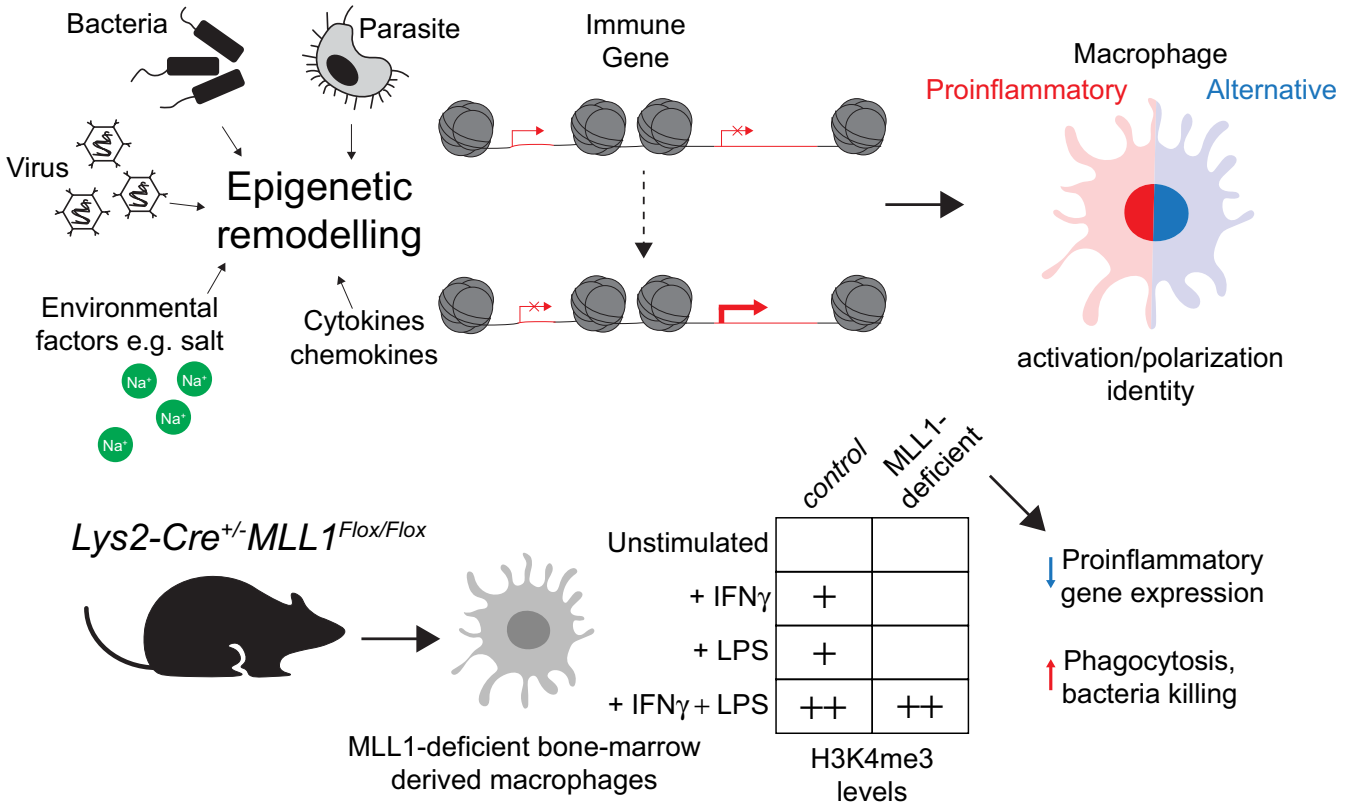

FIGURE 6 Epigenetic remodeling induced by local and systemic challenges tunes macrophage polarization and response. Epigenetic remodeling induced by multiple factors shapes the regulation of macrophage activation, polarization, and cell type identity. Classical proinflammatory activation of macrophage is in part contributed by the activity of the histone methyltransferase. When activated with various LPS/IFNγ, H3K4Me3 levels are increased in controls but not in MLL1-deficient macrophages. Deficiency in methyltransferase led to decreased proinflammatory gene expression but increased phagocytosis and bacteria killing effects

This, in turn, activated *Nfat5*, as a downstream signaling target from the p38/MAPK pathway,[194,195] and expression of *Nos2*, boosting the nitric oxide-mediated Leishmanicidal activity.[193] Conversely, high salt concentration is prohibitive to alternatively activated (M2) macrophage responses, with hypertonicity blunting the activation of interleukin (IL)-4- and IL-13-simulated murine bone marrow-derived macrophages, evidenced by reduced expression of key molecules such as *Arg1* and *Mrc1*.[196] Interestingly, salt augmentation did not simply induce an M1 phenotype in the IL-4- and IL-13-stimulated macrophages; chromatin immunoprecipitation followed by sequencing (ChIP-seq) of the chromatin activating marks, H3K4me3, revealed that IL-4- and IL-13-simulated cells displayed reduced H3K4me3 marks around key M2 signature genes (*Mgl2* and *Slamf1*) in the presence of high salt.[196] This was in contrast to LPS-stimulated macrophages which showed an increased amount of H3K4me3 marks around *Slamf1* and not *Mgl2*,[196] and also around *Nos2*[193] in the presence of high salt. Similarly, expression of Nfat5 was associated with the demethylation of the H3K27me3 modification, a repressive mark when methylated; LPS stimulation was associated with increased chromatin accessibility and increased NFAT5-dependent H3K27me3 demethylation in the promoters of NFAT5-target genes (*Nos2*, *Il6*, and *Tnf*).[197] The distinct patterns in histone modification marks between the macrophage polarization states seem to be a general phenomenon, as Satoh et al[198] observed a similar pattern in murine bone marrow-derived macrophages where they found that Jumonji domain containing-3 (Jmjd3), a H3K27 demethylase that is regulated by TLR-NF-κB (Nuclear Factor kappa-light-chain-enhancer of activated B cells) signaling,[199,200] while not required for M1 macrophage polarization, was critical for M2 responses and defense against helminth infection.

As with development and homeostasis, gene expression during macrophage activation against pathogens is also subject to epigenetic control (Figure 6). Classical activation of macrophages with LPS induces profound epigenetic remodeling[177,184]; this is, in part, contributed by increased expression of the histone methyltransferase, mixed-lineage leukemia 1 (MLL1), and the corresponding increased methylation status of the 4th lysine residue of the histone H3 protein (H3K4).[201] While deletion of MLL1 was associated with a decrease in proinflammatory gene expression, there was a paradoxical increase in phagocytosis and bacteria killing capabilities[201] (Figure 6). It was proposed that these epigenetic mechanisms controlled by MLL1 exist to balance competing immune responsibilities of tissue macrophages, whether or not to participate as a local coordinator of inflammation, or to direct pathogen clearance. In another example, NASH reprograms the KC epigenetic landscape, affecting 18% of enhancer-like regions to either gain or lose H3K27ac marks, with a suggested net effect of transcriptional repression and suppression of genes such as *C6* and *Cd163*, which are important for KC identity, but increased accessibility to genes such as *Trem2* and *Cd9*, which are important for lipid-associated macrophage identity.[186] This was associated with alterations to Liver X receptor (LXR) binding and activity, as genomic regions impacted by the NASH-induced changes to the epigenetic landscape were enriched for LXR response elements/binding motifs.[186]

Similarly, activation of an anti-viral response against Influenza A virus was mediated by type 1 interferon (IFN1)'s regulation of a lysine methyltransferase, SET-domain bifurcated 2 (*Setdb2*), which trimethylates H3K9 (H3K9me3) to silence gene expression[202] in mouse and human macrophages.[203] In contrast to the epigenetic control of proinflammatory response in the earlier example, the effect here is to suppress innate and adaptive response for host protection, limiting collateral damage induced by an overzealous anti-viral response; IFN1 stimulation triggers the interferon regulatory factor 7 (IRF7)-Janus kinase (JAK)-signal transducer and activator of transcription (STAT) signaling pathway and induces the expression of Setdb2, which suppress the expression of genes encoding inflammatory cytokines and chemokines, including *Tnfa*, *Cxcl9*, and *Cxcl10*.[203] Setdb2 was found to bind to the promoters of interferon response genes Isg15 and Mx1, which also contained high signals of H3K9me3.[203] This had a knock-on effect on regulating anti-viral T cell response.[203] Macrophage functions such as phagocytosis could also be regulated by epigenetic modifications; genes related to phagocytosis were found in differentially methylated regions as monocytes develop into macrophages.[204] Unfortunately, while the original purpose of these epigenetic mechanisms may be to promote immune surveillance and pathogen clearance while limiting collateral damage, they can also be exploited for the benefit of pathogens for immune evasion. For example, intracellular parasites such as *Leishmania donovani* can alter the DNA methylation patterns at specific CpG sites in infected macrophages, allowing for the establishment of an intracellular replication niche for the parasite.[205]

Turning to kidney-related injury models, in a mouse model of acute kidney injury (AKI) induced by ischemia-reperfusion, expression of myocardin-related transcription factor A (MRTF-A) recruits the histone acetyltransferase, Myst1 (also known as K(lysine) acetyltransferase 8 (Kat8)),[206,207] to the promoters of NAPDH oxidase family genes and activate reactive oxygen species generation.[208] Macrophage-specific deletion of MRTF-A was sufficient in abrogating the deleterious effects of AKI.[208] These epigenetic effects in renal injury are likely to be cell type- and context-specific; recent studies have also shown that MYST1 plays an essential role in the regulation of transforming growth factor beta (TGF-β) induced autophagy in fibroblasts during fibrosis, which contributes to renal interstitial fibrosis in a unilateral ureteral obstruction (UUO) model,[209] where expression of MYST1 was beneficial, as it limits autophagy and consequently prevent fibrosis[210]; endothelial-specific deletion of Brahma-related gene-1 (BRG1, also known as SWI/SNF related, matrix associated, actin-dependent regulator of chromatin, subfamily a, member 4 (SMARC4)) suppressed UUO-induced renal fibrosis by promoting histone acetylation and methylation of promoters of key genes involved in collagen production, intercellular adhesion molecules 1 (ICAM1), and monocyte/macrophage recruitment (CCL2).[211] microRNA regulation of the macrophage gene expression response during kidney injury has also been reported, in particular, the roles of miR-142 were shown to control macrophage polarization

states and promote macrophage pro-fibrogenic effects.[212,213] Increasing evidence has also suggested that the transition from bone marrow-derived monocytes/macrophages to myofibroblasts (macrophage-myofibroblast transition; MMT) play important roles in the regulation of fibrosis in kidneys.[214,215] Transcriptional control of MMT has been suggested to be governed by POU Class 4 Homeobox 1 (Pouf1), a brain-associated transcription factor, which promotes TGFβ/SMAD's role in mediating MMT and fibrosis.[216] Given that epigenetic modifications regulate multiple facets of TGF-β/SMAD-signaling, fibrosis, and macrophage development, as discussed, a thorough understanding of the epigenetic mechanisms governing MMT could inform therapeutic targeting. Certainly, preclinical models testing the efficacy of histone deacetylase inhibitors on kidney fibrosis and chronic kidney disease have shown promising results.[217-219]

5.2 | Dendritic cells

Dendritic cells (DCs) are professional antigen-presenting cells with key roles in bridging the innate and adaptive immune response. In steady state, DCs arise from HSC progenitors, involving a differentiation route through successive developmental stages, including multi-potent progenitors (MPP) and common dendritic cell progenitor (CDPs), before commitment to mature conventional DC (eg, cDC1 and cDC2) or plasmacytoid DC (pDC) fate.[220] Development of cDC1, cDC2, and pDC requires FMS-like tyrosine kinase 3 ligand (FLT3L) signaling in both mice and humans.[221-225] Of note, human and mouse pDCs have been shown to develop from both myeloid and lymphoid progenitors,[225-229] with different antigen processing and presentation capacity depending on the developmental origins.[230] During inflammation, DCs can also arise from monocytes (generally referred to as monocyte-derived DCs (moDCs)) in both mice and human[231-234] although distinct 'inflammatory DCs' (referred to as cDC3), have also been reported to develop from granulocyte-macrophage progenitors (GMP) in humans,[235,236] dependent on granulocyte-macrophage colony-stimulating factor (GM-CSF) rather than FLT3L signaling.[235] In vitro generation of DCs or macrophages from human monocytes could be achieved via supplementation with GM-CSF and IL-4 or GM-CSF alone, respectively[237]; IL-4 signaling activates downstream JAK3-STAT6 signaling, generating immature DCs.[238] Using genome-wide DNA methylation bead arrays for >450 000 CpG sites, it was found that methylcytosine dioxygenase (TET2)-dependent demethylation of DC-specific genes, after IL-4 signaling and activation of JAK3-STAT6 pathway, was critical in ensuring DC development from monocytes.[239] Inhibition of STAT6 phosphorylation (active STAT6) resulted in a methylome that was similar to macrophages, preventing proper DC development from monocytes.[239] While there were no detected direct interactions between STAT6 and TET2, it was postulated that a recruitment relay between STAT6, PU.1, and TET2[240,241] could be a possible mechanism for STAT6-dependent regulation of DC-specifying DNA methylation. Other DC populations such as AXL^+ DCs have also been discovered with recent advances in single-cell techniques in humans,[242,243] as well as in mice.[244] The exact functions and ontogenies of these new DC subsets remain an open area of research.

Transcription factors such as PU.1, Core-Binding Factor Subunit Beta/Runt-related transcription factor (CBFβ/RUNX), Basic Leucine Zipper ATF-Like Transcription Factor 3 (BATF3) are required for regulating the expression of IRF8, a key DC lineage-defining transcription factor, and are expressed during the specific stages of DC development.[245-247] The expression patterns of these lineage-defining transcription factors also coincide with the acquisition of relevant activating or repressive histone marks across the differentiation stages of DC fate commitment.[248,249] For instance, IRF8 expression in lymphoid-primed multi-potent progenitors (LMPP) and monocyte-DC progenitors (MDP) primes the development of cells into DCs, linked to increased chromatin accessibility to enhancer elements for DC-related genes.[250,251] Similarly, H3K4me3 (gene activation) and H3K4me1 (primed enhancers) histone marks in the progenitor stages of MPP and CDP were restricted to progenitor genes including *Cebpb*, *Id1*, *Gata2*, and *Myc* while mature DCs and pDCs displayed enrichment of these marks in *Batf3*, *Id2*, *Irf5*, and *Irf8*.[249]

Concomitantly, H3K27me3 (gene repression) marks were observed to be enriched for the progenitor-associated genes in cDCs and pDCs and vice versa, that is, the repressive histone marks were observed in cDC- and pDC-associated genes in progenitor cells.[249] The authors posit that epigenetic feedback loops stabilize the transcriptional program during pDCs development to ensure the developmental route; while the promoter and enhancer regions of cDC factor such as Irf4 are regulated by PU.1 and Irf8 and Stat3 binding, including auto-regulatory loop for Irf4 itself, they were not observed to form feedback loops, unlike pDC factors such as *Ets1*, *Irf1*, *Spib*, and *Tcf4*.[249,252,253] Similarly, in a separate ChIP-seq study comparing moDCs versus pDCs, while there was relative small number of differentially enriched activating promoter H3K4Me3 marks, there was a substantial differential usage of H3K4me1 and H3K27ac enhancer marks between the two cell types.[254] In particular, they found that Irf8 and Cebpb bound more regions and pDC- or moDC-specific H3K4Me1 enhancer regions in pDC and moDC respectively.[254] shRNA knockdown and overexpression systems of Irf8 or Cebpb skewed the expression of pDC- or moDC-related genes, respectively. This was further supported by reciprocate accumulation of H3K4me1 marks in moDC-specific gene regions, and reduction in pDC-specific gene regions, when Irf8 was knocked down in pDCs, and *vice versa* in moDC when Cebpb was knocked down.[254]

As observed in macrophages, the epigenome of DCs can be further altered during activation or infection. For instance, infecting ex vivo differentiated human moDCs with live *Mycobacterium tuberculosis* (MTB) resulted in a general decrease in DNA methylation status (whole-genome shotgun bisulfite sequencing).[255] Although only a small percentage of hypomethylation events were situated near a promoter region, with the majority in intergenic or intronic regions, a large proportion of regions were situated within H3K4me1 primed enhancer regions already present in non-infected DCs.[255] Live MTB infection increased association with active H3K27ac marks and

decreased association with H3K4me1 (primed) marks.[255] Infection was associated with increased chromatin accessibility of active enhancer marks, as well as increased genome-wide binding of NF-κB and IRF transcription factors in infected DCs.[245] However, a subsequent study in the same DC infection model found that changes in gene expression were not entirely dependent on methylation status,[246] suggesting that they play a role in fine-tuning immune responses or even innate immune memory.

During autoimmune disease, for example in lupus nephritis, circulating DCs ($CD1c^+$ moDCs and pDCs) showed increased mRNA expression of *IRF1* and *IRF8* in disease compared to control, in moDCs and pDCs respectively.[257] There was a trend of genome-wide hypermethylation in moDCs in severe disease (assessed by global increase in 5mC percentage) and a decrease in genome-wide trimethylation status of H3K4me3 and H3K27me3 histone marks in moDCs during milder disease.[258] While these transcriptomic and epigenetic changes are supportive of potential roles for epigenetic alterations in DC in kidney disease, little is known of their status within the actual tissue/organ; DCs in kidneys are a heterogenous population of cells, sharing many markers with macrophages, and making the study of DCs in kidney health and disease difficult.[26,259] We, and others, have been able to use single-cell transcriptomics to profile the kidney immune landscape, finding distinct clusters of cells that resemble cDC2 and pDC[26,259]; consistent with other human tissues,[260] cDC1s are detectable but rare in our kidney scRNAseq data. Future efforts in this space would hopefully include multi-omic single-cell techniques to hopefully address the important unanswered questions about how tissue DC gene transcription is controlled in health and disease.

5.3 | Trained immunity

The classical view of innate versus adaptive immunity is that immunological memory is a unique, hallmark feature of the adaptive response, exemplified by the acquisition of distinct memory states in B cells and T cells, and the ability to generate rapid, antigen-specific responses to subsequent challenge. However, recent studies have supported the concept of "trained immunity," the ability of innate immune cells, such as macrophages and DCs, to adopt adaptive traits, where responses to re-infection/re-challenge are influenced by a previous exposure.[261] A classic example is a phenomenon known as LPS tolerance, where LPS pre-treatment can induce a transient state in macrophages, such that they become unresponsive or weakly responsive (refractory) to further stimulation with LPS[262,263] (Figure 7). The tolerant state is, in part, mediated by epigenetic changes that alter gene expression patterns[184,264]; the epigenetic landscape of LPS-tolerized macrophages differs from that of naive macrophages, as well as that of LPS-activated macrophages, with a loss of H3K27ac deposition at promoters and enhancers of specific immune-related genes evident.[184,265] Beta-glucan (β-glucan), a component of fungal cell wall, can be used to "re-train" LPS-tolerized macrophages, reversing the epigenetic modifications induced by LPS, and restoring macrophage production of LPS-induced cytokines.[265] Other studies have shown that exposure to *Candida albicans* or β-glucan can prime and enhance the inflammatory response in macrophages toward other non-related pathogens.[266] A more thorough understanding of the effects of LPS tolerance and its reversal may have important consequences on informing therapeutic strategies to reduce sepsis mortality, given the limited success of anti-inflammatory agents for treating sepsis,[267] and the possibility that macrophage tolerance may contribute to the immunosuppressive state and increased risk of secondary infections associated with sepsis.[268]

5.4 | Tissue-resident T cells

Tissue-resident memory T cells (Trm) represent a subset of T cells which occupy tissues and do not recirculate, forming a separate pool distinct from T cells in peripheral blood or secondary lymphoid organs.[269] These cells perform surveillance and rapid effector functions in tissues, at the site of initial antigen recognition. They have been well characterized at mucosal and barrier surfaces, but also

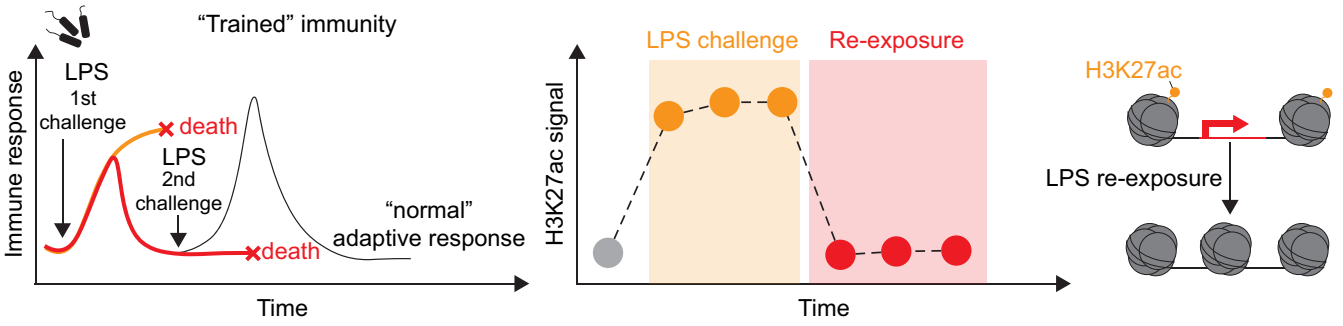

FIGURE 7 "Trained" immunity and consequences of LPS tolerization. In contrast to traditional adaptive response, trained immunity refers to a memory-like response displayed by innate immune cells such as monocytes, macrophages, and DCs. An example is LPS tolerance, a phenomenon characterized by a diminished response upon subsequent LPS challenge. This is thought to have important consequences in sepsis, which can cause death either due to an initial hyperactivation of the immune system and induction of cytokine storm or a delayed immunosuppressive state characterized by secondary infections that is also associated with increased mortality. The suppression of the immune response is in part attributed to epigenetic modifications in the cells, leading to a loss of H3K27ac deposition in important genes, suppressing gene expression

exist in non-barrier tissues in mice and humans. They are influenced by local environmental factors particular to the tissue in which they reside. These include oxygen tension, paracrine signaling from parenchymal cells and other leukocytes, mechanical and chemical cues from extracellular matrix, and direct cell-cell interactions. While most studies have described a role for Trm in defense against infection, they may play roles responding to other excursions from tissue homeostasis.[10,269]

Trm persist at sites of microbial exposure, and act as readily activated sentinels, poised to generate responses to previously encountered antigens. Schenkel et al[271] have shown that local re-challenge at mucosal surfaces (in this case the murine female reproductive tract), elicits rapid elaboration of inflammatory chemokines such as CCL3 and CCL4 by Trm and CXCL9, CXCL10, and CX3CL1 from neighboring endothelial cells.[271] Furthermore, evidence from the female reproductive tract suggest $CD4^+$ Trm are maintained by chemokine signaling from local tissue-resident macrophages.[12]

The skin is another well studied organ, establishing several key concepts in Trm biology. Trm in the skin are able to initiate very rapid recall responses to specific pathogen antigens. Vaccinia virus (VACV) skin infection establishes residency of $CD8^+$ T cells, which in contrast to the circulating $CD8^+$ central memory T cells (Tcm), produce cytokines, persist in the skin after infection, and augment viral clearance.[29] These $CD8^+$ $CD103^+$ Trm develop from T cells infiltrating the skin, and are directed by IL-15 and TGF-β signaling.[270] The metabolic programme of these cells becomes reshaped as they become skin-resident, with upregulation of lipid uptake apparatus (*Fabp4* and *Fabp5*), with increased free fatty acid uptake and mitochondrial oxidative phosphorylation, enhancing cell survival. These metabolic features are observed in murine and human skin, suggesting that reprogramming of metabolism is a core adaptation supporting Trm function.[272]

The transcriptional basis of the T cell tissue residency programme has been investigated using bulk RNA-seq of sorted Trm and NK cell populations across multiple organs (including skin, liver, gut, and kidney) in the mouse. Mackay et al[13] uncovered specific upregulation of the transcription factors Hobit and Blimp1 mediating the enforcement of the tissue residency programme. Indeed ChIP-seq of Blimp1 and Hobit interacting genes highlighted repression of genes encoding proteins involved in lymphocyte egress such as *Ccr7* and *S1pr1*. Building on these findings, Parga-Vidal et al[273] used Hobit reporter mice, finding $Hobit^+$ effector T cells are biased toward Trm fate during the immune response to LCMV infection. However, the human system may exhibit important differences in relation to the transcription factor circuitry enforcing tissue residency—transcriptional profiling of human lung $CD103^+$ Trm demonstrated transcription of chemokine receptor and adhesion molecule genes consistent with a tissue residency programme, but the transcription factor programmes driving Trm identity included NF-κB complex components and Notch1-RBPJ, with no Hobit/Blimp1 signal evident.[274] Analysis of human $CD69^+$ $CD4^+$ and $CD8^+$ Trm with bulk RNA-seq has identified core transcriptional signatures associated with Trm status. Consistent with previous data, *ITGAE* (CD103), *ITGA1* (CD49a), *CXCR6*, and *CX3CR1* form key upregulated components of this signature, whereas lymph homing apparatus genes such as *S1PR1*, *KLF2*, and *SELL* are all downregulated. While core signature components are similar to those seen in the murine system, *ZNF683* (Hobit) is not a differentially transcribed gene in the human data, suggesting distinct control mechanisms for Trm establishment and maintenance in the human system.[14]

In contrast to upregulated transcription factors enforcing a Trm state, downregulation of the T-box transcription factors T-bet (*Tbx21)* and Eomes has been shown to play an important role in the formation of murine $CD8^+$ Trm. These transcription factors are coordinately downregulated during Trm generation, and T-bet knockouts show enhanced Trm formation. Importantly TGF-β appears to be reciprocally regulated with respect to T-bet, suggesting that as T cells acquire signals directing their differentiation toward a Trm fate, their transcription factor circuitry and perhaps their epigenome is concurrently reprogrammed to further enhance the establishment of residency.[275]

In the kidney, local TGF-β promotes the formation of Trm through enhancing the entry of effector memory T cells (Tem) into the tissue,[276] consistent with similar mechanisms acting in the intestinal epithelium and skin.[270,277] Furthermore, downregulation of the IL18 receptor, promoted through TGF-β signaling appears to be an important step in establishing T cells with a cell surface marker profile consistent with residency ($CD103^+$ and $CD69^+$), and an upregulated transcriptional residency programme in the kidney.[278] While in mice, TGF-β has been identified as a signal influencing the differentiation of kidney-resident Trm, the environmental signals controlling the retention and maintenance of transcriptional and epigenetic programming of these cells remains to be uncovered.

It is clear that Trm cells have a distinct transcriptional programme; however, the epigenetic mechanisms maintaining and guiding this state continue to be investigated and refined. Using an RNAi in vivo screening methodology in the context of murine LCMV infection, Milner et al[279] compared Trm in the kidney and small intestinal intraepithelial lymphocytes to circulating and splenic effector T cell populations. Using both RNA-seq and ATACseq these authors found these populations to be transcriptionally and epigenetically distinct, with splenic populations highly expressing the transcription factors *Eomes* (Eomesodermin) and *Tbx21* (T-bet), in contrast to high expression of *Prdm1* (Blimp1), *Nfkb1*, and *Nr4a1* in kidney Trm. Integrating data on transcription factor binding motif accessibility and transcription factor expression in the context of a loss-of-function screen, these authors identified the transcription factor *Runx3* as a key regulator of $CD8^+$ Trm differentiation and maintenance. Indeed using tamoxifen-inducible deletion, they found *Runx3* deficiency resulted in substantial loss of Trm in the kidney, skin, and lungs. *Runx3* appears to be an important transcriptional regulator of kidney-resident Trm; in a murine allograft model, scRNAseq of infiltrating T cells identified Trm. These cells showed strong expression of the transcription factors *Runx3*, *Nr4a1*, *Prdm1*, and the lipid transporter gene *Fabp5*.[280]

While several investigators have attempted to define the epigenetic and transcriptional programmes at work in Trm across numerous

organs, tissue and microenvironmental specific influences of Trm molecular phenotypes are less well understood. Exploring distinct anatomical compartments of the lung, Hayward et al[281] used bulk RNA-seq contrasting murine airway Trm, lung interstitial Trm and splenic Tem. While interstitial Trm and airway Trm shared a core tissue residency signature including the genes *Itgae*, *Ahr*, *Cxcr6*, *and S1p1r*, they found an enriched unfolded protein response specifically in airway Trm suggesting an adaptation to the unique environment of the airway. They then turned to bulk ATACseq of these populations to define the epigenetic programmes imbued by the tissue environment, finding a unique chromatin accessibility pattern in airway Trm enriched for binding motifs of STAT5 and DNA Damage Inducible Transcript 3 (DDIT3). In contrast interstitial Trm had an enrichment pattern characterized by FOS and cAMP Responsive Element Modulator (CREM) motifs. Connecting the epigenetic profiles and transcriptional profiles, the authors found the airway Trm chromatin accessibility signature was enriched for genes involved in endoplasmic reticulum (ER) stress and glucose starvation, consistent with the unfolded protein response uncovered by their RNA-seq analysis. Together, their results suggest that the tissue location and environment can uniquely programme the epigenome, and as a result, the transcriptional programmes activated in Trm.

Exploring the behavior of Trm during recall responses, Fonseca et al[282] show that Trm are able to egress into the circulation following restimulation. Using WGBS, they profiled DNA methylation in naive and activated T cells, and memory subsets; Tcm, Tem, and Trm. Analysis of genome-wide CpG methylation indicated memory subsets exhibit similar patterns of CpG methylation, distinct from naive and activated subsets, and showed Trm exhibit a moderate degree of plasticity when compared to either naive or exhausted T cells suggesting they may not be terminally differentiated. Indeed these authors find that Trm are able to transdifferentiate into circulating memory T cells months after LCMV infection. Transfer of Trms which have egressed and transdifferentiated, into naive recipient mice which are subsequently challenged with LCMV, showed that these cells readily re-enter tissues and re-establish residency. Together these findings suggest epigenetic programming is able to balance lineage commitment and plasticity, permitting activation and mobilization of Trm and re-establishment of the Trm phenotype over the time course of an infection.

5.5 | Natural killer cells

Natural killer (NK) cells are innate lymphoid cells best known for their cytotoxicity, identifying and killing virally infected or malignantly transformed cells that have downregulated MHC class I. In humans, NK cells consist of two major subsets—$CD56^{dim}CD16^{+}$ and $CD56^{bright}CD16^{-}$, the former being the dominant circulating subset that exhibits high cytotoxic capacity.[283] NK cells with a tissue-resident signature have been identified in a number of organs, including spleen, lymph nodes, and liver, and largely consist of the $CD56^{bright}$ subset, characterized by lower cytotoxicity and higher cytokine production, including IFN-γ and TNF-α, stimulating macrophage antimicrobial functions.[284] NK cells express the lineage transcription factor marker EOMES[285]—which is essential for NK cell differentiation - and distinct phenotypic markers such as Killer Cell Lectin Like Receptor F1 (KLRF1/NKp80), and cytotoxic effector molecules such as granzymes and perforin.[285, 286]

Epigenetic modifications through DNA methylation, histone modification, as well as non-coding RNAs are critical for NK cell differentiation. The maturation of NK cells is characterized by the decreasing levels of DNA methylation at the promoter regions of IFN-γ, Killer Ig-Like Receptors (KIRs), and CD94/NK group 2 member A (NKG2A), resulting in the upregulation of their transcription critical for cytotoxicity. In early hematopoietic progenitor cells, methylation of the promoter regions of KIR genes by DNMT1 leads to KIR gene silencing.[287] Upon differentiation and maturation, the chromatin structure opens through critical histone modifications at H4K8ac,[288] and KIR genes are actively transcribed. In addition to the role of DNA methylation in NK cell differentiation, histone modifications, mainly histone acetylation and methylation, determine NK cell activation and effector functions in the context of infection. Constitutive Ifng expression is critical for NK and NK T cell development and identity.[289] By comparing the histone hyperacetylation patterns in *Ifng* between T cells and NK cells, Chang et al[290] found that histones of the *Ifng* gene region were acetylated in steady state, irrespective of the NK cell activation, providing mechanistic insights into the capacity of NK cells for rapid cytokine responses. In addition, NK cell activation and cytokine signaling are associated with redistribution of p300 recruitment and key transcription factors, including STAT and T-bet, which contribute to rapid induction of effector genes.[291] Similar to *Ifng*, *NKG2D* also had a high level of H3K9ac and a low level of H3K4me3, suggestive of active transcription. Lastly, key miRNAs have been identified in the development, maturation, and effector functions of NK cells.[292] These miRNAs play a repressive role in regulating target gene expression, including those encoding perforin (miR-30e, miR-150), granzyme B (miR-378), and IFN-γ (miR-146a; via NF-κB signaling).

5.6 | Structural cells

While not traditionally considered to play a part in immune responses, increasing evidence supports the conclusion that non-immune cells that exist alongside specialized immune cell subsets within tissues work in concert with professional immune cells to maintain organ health. For example, in the kidneys, epithelial cells in different anatomical regions show distinct immune functions; pelvic epithelial cells, the first kidney cells encountered by bacteria ascending from the bladder, basally express antimicrobial peptides such as lipocalin 2 (LCN2), an iron chelator that has roles in limiting bacterial growth[293] and is associated with prevention of urinary tract infections,[294] and neutrophil- and MNP-recruiting chemokines, such as *CXCL8* and *CX3CL1*,[26] in homeostasis, suggesting they are primed to respond to infectious challenge. In contrast, podocytes show a different chemokine expression profile, with high expression

of *CXCL12*,[26] with the capacity to attract CXCR3/4-expressing cells, and have been shown to express MHC-II molecules during inflammation.[295]

Recently, a multi-omic analysis of endothelial cells, fibroblasts, and epithelial cells across twelve different murine organs revealed organ-specific epigenetic and transcriptomic networks that supported immune activity and homeostasis.[27] The structural cells expressed receptors and ligands enabling interactions with immune cells in an organ- and cell type-specific manner; for example, interactions between endothelial cells with NK cells or monocytes were particularly enriched in kidneys.[27] Specific immune-related genes expressed in kidney endothelium, epithelium, and fibroblasts in homeostasis included *Lrp2* (lipoprotein receptor-related protein 2), *Spp1* (encoding osteopontin (OPN)), *Dysf*, and *Col4a3* (collagen type IV alpha 3 chain), some of which have disease associations (Figure 8A); for example, *Lrp2* encodes megalin, an endocytic receptor for filtered protein and defects in this protein can cause tubular proteinuria and anti-LRP2 is a model for nephritis called Heymann nephritis[296] and was identified in predicted interactions between kidney endothelium, epithelium, and fibroblasts with monocytes, B cells, and macrophages.[27] Furthermore, OPN receptors include some integrins and CD44, expressed by many immune cells, and OPN-deficient mice demonstrate more severe kidney damage in the nephrotoxic nephritis model of immune complex-mediated glomerulonephritis.[297]

The epigenetic landscape of structural cells in this pan-organ study was consistent with the transcriptomic findings, showing open chromatin accessibility profiles in many important genes and transcription factors, including the promoter region of *Cdh16* as a kidney-specific effect in the three cell types examined. Importantly, regions around immune-related genes were also observed to be characteristically open in homeostasis, including regions near *Tlr9* in the brain and liver, and *Stat5a* and *Stat5b* in the heart, intestines, and spleen; high chromatin accessibility for *Ifngr1* promoter region was observed in the brain, caecum, spleen, heart, kidneys, and liver[27] (Figure 8B). This supported the conclusion that the structural cells adopt a primed epigenetic state enabling rapid future immune activation. Indeed, in both lymphocytic choriomeningitis virus (LCMV) infection and following cytokine challenge, the organ structural cells displayed a cell type- and organ-specific activation of immune response genes and pathways.[27] By contrasting the mRNA expression post-challenge with that of the RNA-expression profile and epigenetic profile of cells in homeostasis, a substantial number of the immune genes were found to be initially lowly expressed in homeostasis but were "poised" for immune activation, with higher chromatin accessibility[27] (Figure 8B). The effects of cytokine stimulation in the kidney were not specifically investigated or functionally validated due to the relatively weak effects of LCMV challenge on structural cell transcriptomes compared to other organs, but it may

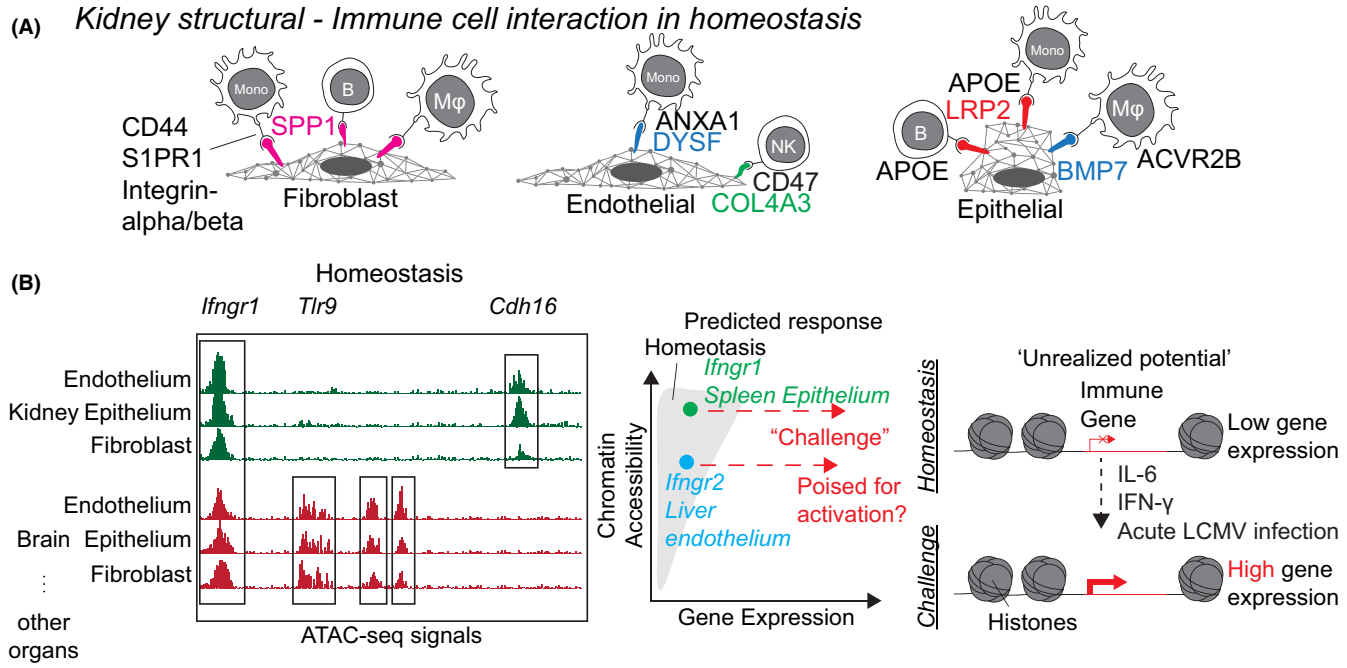

FIGURE 8 Epigenetic modification of non-immune cells primes regulation of tissue immunity. A, In the kidney, potential interactions between fibroblasts, endothelial cells, and epithelial cells with monocytes, macrophages, and B cells are predicted from the transcriptome data, with molecules such as *Lrp2*, *Spp1*, *Bmp7*, and *Apoe* being expressed on the non-immune cells. B, The transcriptomics findings were correlated with increased chromatin accessibility profiles in a cell-type and organ-specific manner. For example, the increased accessibility to the promoter region of renal adhesion molecule *Cdh16* was exclusive to kidneys. Importantly, increased chromatin accessibility was observed for regions related to several immune genes including *Ifngr1* and *Tlr9* during homeostasis. Many immune response genes were "poised" for activation in non-immune cells, characterized as displaying increased chromatin accessibility but low gene expression during homeostasis and high gene expression after challenge. Adapted from Krausgruber et al[27]

well be that "primed" gene expression in kidney structural cells may play a role in immune responses to renal injury and infection models.

While this large resource sheds new light on the underappreciated role of "structural immunity," there is likely underlying heterogeneity within cell subtypes that remains to be validated, exemplified by our single-cell characterization of the non-immune landscape in the kidney,[26] and by others across a variety of tissues and organs. Nevertheless, we anticipate future efforts to extend to single-cell resolution, for example, the human cell atlas of fetal transcriptome[139] and epigenome,[298] mapping a more complete understanding of the regulation of immunity.

6 | CONCLUSIONS AND FUTURE DIRECTIONS

Epigenetic profiling of tissue immune cells has revealed some of the details of the mechanisms that control the development, recruitment, maintenance, and activation of these cells. The best-studied tissue cell is the macrophage, with detailed knowledge of the epigenetic mechanisms that control their development in embryogenesis, their recruitment and tissue specification, their replenishment from the monocyte pool, and their polarization and activation. As we increasingly appreciate the diversity of immune cells present within organs beyond macrophages, it is evident that much more work is needed to define the mechanisms at play in controlling the transcriptional activity of these cells, along with organ structural cells to generate coordinated, tissue- and stimulus-specific immune responses. Continuing technological advances should enable the acquisition of this information at single-cell resolution, and their application to human tissue samples in health and disease, including in the kidney, has the potential to identify cell- and tissue-specific epigenetic targets for disease treatments across organs.

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CONFLICT OF INTEREST

All authors declare that potential conflicts do not exist.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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