**Writ large: Genomic dissection of the effect of cellular environment on immune response**

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Cells of the immune system routinely respond to cues from their local environment and feed back to their surroundings through transient responses, choice of differentiation trajectories, plastic changes in cell state, and malleable adaptation to their tissue of residence. Genomic approaches have opened the way for comprehensive interrogation of such orchestrated responses. Focusing on genomic profiling of transcriptional and epigenetic cell states, we discuss how they are applied to investigate immune cells faced with various environmental cues. We highlight some of the emerging principles on the role of dense regulatory circuitry, epigenetic memory, cell type fluidity, and reuse of regulatory modules in achieving and maintaining appropriate responses to a changing environment. These provide a first step toward a systematic understanding of molecular circuits in complex tissues.

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**Fig. 1. Key modes of immune-environment interaction.** (A) Transient responses to signals. A cell responds to an environmental signal (lightning bolt) with a short-term, transient response (marked time points). (B) Balanced differentiation along hematopoiesis. Shown is a schematic of a lineage tree. Solid arrows, individual (known) differentiation steps; dashed arrows, multistep process. (C) Stable yet plastic cell-type polarization. After polarization (solid black arrows) from a progenitor cell (e.g., naïve T cell; gray circular cell), cells with different differentiated states (colored cells) can plastically transition (dashed gray lines) to other states under appropriate signals—for example, from other immune cells (e.g. dendritic cell; large tentacle gray cell). (D) Malleable adaptation of tissue-resident cells. A cell of a single type (e.g., macrophage, gray; T cell, blue) can reside long term in different tissues (gray), assuming unique characteristics stereotypical to that tissue.
Continuous emphasis on the regulation of mRNA expression, especially transcriptional and epigenetic profiling. Using macrophages and T cells as case studies, we discuss the types of features that are underscored with different genomic profiles, the resulting testable hypotheses that can be followed up in dedicated low-throughput experiments, and the emerging organizing principles and mechanisms (Fig. 3). Finally, we discuss how these lessons, learned in immune cells, can be extended to develop approaches to dissect the overall function of diverse cells in maintaining homeostasis.

Molecular responses of the immune system through transient intracellular circuits

Macrophages, innate immune cells of the myeloid lineage, serve key immune defense functions through phagocytosis and by communicating with adaptive immune cells through antigen presentation and secretion of and response to cytokines and chemokines (22–24). Mononuclear phagocyte subpopulations are located in the circulatory system and spleen and can differentiate into macrophages, but macrophages also reside in other tissues, where they acquire specific characteristics and contribute to local hemostasis (1, 22, 23).

In their role as immune sensors, macrophages express pattern-recognition molecules [e.g., Toll-like receptors (TLRs)], which detect conserved pathogen-associated or tissue damage-associated molecular patterns, and mount the appropriate response. Genomic analysis of macrophages provided key insights on how these transient responses (Fig. 1A) are carried out. For instance, transcriptional profiling along a time course after TLR4 activation with bacterial lipopolysaccharide (LPS), an inducer of inflammation, showed that genes are induced in several consecutive “waves” (23), a phenomenon observed in many other response systems (26) (Fig. 3). The response waves are regulated through successive activation of transcriptional regulators (27, 28), whose identity can be predicted from DNA-binding motifs enriched in the 5’ regions of the responding genes. This approach helped identify activating TF 3 (ATF3) as an early regulator of the LPS response in macrophages (25). ATF3 was then shown to recruit histone deacetylase to repress its target genes, thus forming a negative feedback. This mechanism may be essential for controlling the extent and duration of TLR-induced inflammation during infection, avoiding rampant inflammation and tissue damage.

The importance of epigenetic regulation as a way of controlling macrophage activation was further demonstrated by investigating the formation of “memory” in antigen-exposed macrophages (Fig. 3), where macrophages repeatedly exposed to a specific component (e.g., LPS) become tolerant and selectively produce antimicrobial, but not pro-inflammatory, signals to avoid tissue damage (27, 28). Profiling of gene expression and histone modifications (Fig. 3) during the macrophage response to repeated stimulation reveals that “tolerant” genes that are not reinduced in repeated exposure to LPS are enriched for pro-inflammatory functions and are transiently silenced by loss of activating histone marks. Conversely, nontolerant genes are enriched for antimicrobial functions and are associated with a faster and stronger transcriptional response upon LPS restimulation (compared with the primary stimulation) through persistence or rapid acquisition of activating histone marks (H3 trimethylation and H4 acetylation, respectively) and recruitment of RNA polymerase II (Pol II) and chromatin remodeling complexes. These distinct epigenetic mechanisms depend on the protein products generated during the first exposure to LPS, emphasizing the common role of negative feedback in controlling innate immune response (26, 29) (Fig. 3).

Depending on their tissue of origin and stimulus, macrophages can acquire distinct functional states. Two well-studied states are proinflammatory M1 cells, derived in the presence of interferon gamma, and immunosuppressive M2 cells that can be induced with interleukin-4 (IL-4) or IL-13 (30). Profiling the transcriptional response of monocyte-derived macrophages to a more diverse set of stimuli suggests that macrophages can mount diverse transcriptional programs beyond these two states, depending on the metabolites, cytokines, or ligands to which they are exposed (30). Computational analyses of gene modules that are coregulated across programs suggested that diversity between programs is generated by different combinations of active transcriptional regulators (Fig. 3). Some of these regulators are “reused” across all programs (e.g., the lineage-specifying factor PU.1), whereas others are important only in certain contexts (e.g., STAT1 and STAT6, in the interferon gamma and IL-4 responses, respectively). Mapping the diverse activation programs also provided a way to decompose bulk samples into constituent responses—for instance, proposing that alveolar macrophages from chronic obstructive pulmonary disease patients are depleted in the inflammatory (M1) state, which may explain their poor response to anti-inflammatory therapeutics. Such analysis could further benefit from application of single-cell genomics (Fig. 3).

Systematic perturbations have helped establish the causality of circuits inferred from genomic profiles. Causal loci were discovered either by associating natural genetic variation with variation in the transcriptional response across human individuals (16, 18, 19) or mouse strains (17) or by perturbing genes and measuring the effect on the transcriptome (15, 29).

Balanced differentiation from progenitor cells

The diverse cell types of the hematopoietic system are organized in a taxonomy of different lineages...
and are produced daily from a small pool of stem cells (Fig. 1B). The composition of hematopoietic cell subsets is tightly controlled, ensuring both homeostatic control and responsiveness to environmental cues. As in studies of transient immune responses, genomic and epigenomic profiling has shed light on the transcriptional shifts during hematopoiesis and the regulatory programs that govern them (Fig. 3), primarily focusing on unperturbed, homeostatic conditions in humans (2, 31) and mice (14, 32). Transcriptional profiling revealed substantial expression changes between hematopoietic cell subtypes, comparable to those between different tissues (2). Computational analysis of these data, focused on “modules” of coregulated genes and the regulators associated with them, has identified global organizing principles in hematopoiesis that may also apply more generally (Fig. 3).

First, a large set of predicted transcriptional regulators (across all hematopoietic lineages) form a dense interconnected network of regulatory interactions in each cell type and with the same regulator used in multiple hematopoietic subsets. This organization may confer robustness but could also be liable to dysregulation and cancer (2). This model challenged and expanded an earlier hierarchal model of hematopoiesis controlled by a small number of TFs, expressed sequentially (33).

Furthermore, there is no simple partitioning of regulatory activity at different lineages. Instead, entire modules of coregulated genes, along with their upstream regulators, are reused across distinct lineages, either because of shared functional needs in otherwise different cells (e.g., mitochondrial and oxidative phosphorylation in erythroid progenitors, granulocytes, and monocytes (2)) or due to shared developmental descent. This latter pattern is often reflected in “transitional” cases (Fig. 3), with a gradual onset and offset of programs along the hematopoietic cell hierarchy (2). The transitional gene modules could be explained either by the presence of cells at different phases of development within seemingly pure populations of progenitor cells or because regulatory programs of a more differentiated state are fore-shadowed by preexisting programs at earlier stages. Both models are plausible and nonexclusive, and the second model is strongly supported by profiling of TF binding in humans (2) and of chromatin organization in hematopoiesis in mice (14) and humans (33), where a large portion of the enhancers exhibited a “transitional” behavior—already established in the precursor cells, possibly in a poised (and transcriptionally inactive) state. Single-cell RNA-sequencing (RNA-seq) studies can help further address how transition to multiple lineages is concomitantly encoded in progenitor cells. To date, some studies suggested that there may be distinct subsets within myeloid progenitor cells that are partially skewed toward distinct functional fates by the expression of key sublineage regulators (34), whereas others emphasized evidence for obligatory mixed-lineage states within the same single progenitor cell (35).

While it is tempting to think of hematopoiesis as stereotypic, differentiation is affected and driven by the environment, including not only the stromal niche and other immune cells but also stress and pathogens (27, 36). For example, stress signals can lead to production of more innate immune myeloid cells at the expense of other lineages, especially lymphoid cells (36). Furthermore, distinct subpopulations of stem and progenitor cells can be activated (37) to produce cytokines that affect core immune responses. Genomic analysis, including at the single-cell level, will shed more light on the regulation of hematopoiesis by such signals.

**Plasticity of cell differentiation**

As immune cells become more committed, differentiation and balancing between subtypes become even more intertwined with environmental responses. For example, naïve T helper (T<sub>H</sub>) cells can differentiate into multiple specialized cell types, including conventional (T<sub>con</sub>) cells (e.g., T<sub>H1</sub>, T<sub>H2</sub>, T<sub>H7</sub>, and T<sub>H9</sub>) and regulatory T<sub>H</sub> cells (e.g., T<sub>reg</sub> and T<sub>17</sub>) (4). Given the diverse and partially opposing functions of different T<sub>H</sub> cells (4), it is critical to maintain their correct blend in a manner sensitive to and controlled by environmental signals. First, the relative proportion of T<sub>H</sub> subtypes that will develop from a limited pool of naïve T<sub>H</sub> cells is regulated by the blend of cytokines to which a naïve cell is exposed, often produced by antigen-presenting cells (e.g., to T<sub>reg</sub> cells in the presence of transforming growth factor–β but skewed to T<sub>H1</sub> cells if IL-6 is also present). Second, while differentiated T<sub>H</sub> cell subtypes can be maintained stably over time, including in the memory pool (38), some can also transition into other, parallel subtypes (Fig. 1C), depending on extracellular signals, from cytokines, to oxygen or nutrients (39), to components of the microbiome (3). The process leading to these diverse types is often called polarization, rather than terminal differentiation, and the change between the types is referred to as plasticity (1, 4) and can play critical physiological roles. For example, plastic polarization of tissue-resident macrophages helps fulfill changing functional demands from the tissue in which they reside (1, 40); T<sub>H1</sub> cell plasticity could allow an organism to respond to a changing environment even if cells were originally committed to the memory pool in a different state (20).

The distinction between a permanent and plastic state can be defined in principle but is challenging to identify in practice (41) because it can be hard to determine whether a stable state is permanent and whether concomitant expression of markers of different cell type is not mere noise (41). Genomic profiling coupled with lineage tracing and functional studies were instrumental in addressing this question (Fig. 3).

Specifically, using the RNA profile of the cell as its functional identity and coupling it to lineage tracing has helped identify both how the state of TH cells is stably maintained and when it shifts plastically ([20, 38, 42], reviewed in (4)). For example, T<sub>H17</sub> cells can begin to express both cytokines and seminal TFs of other T<sub>H</sub> cells (20, 38) but these could reflect either transition to another type or a transient functional deviation. Lineage tracing of T<sub>H17</sub> cells in the gut followed by RNA-seq showed that they can adopt a transcriptional signature of regulatory T cells and anti-inflammatory capacity (20). Conversely, tracing T<sub>H17</sub> cells in a melanoma mouse model showed that, although they can acquire transcriptional features of T<sub>H</sub>1 cells, they remain distinct from similarly traced T<sub>H</sub>1 cells, acquiring stem-cell–like signatures and longevity, with increased tumor eradication capacity (38).

Although these studies profiled cell populations defined by cell surface, cytokine, or TF expression, recent single-cell genomic studies (6, 43) have increased the resolution at which we characterize cellular subtypes and their fluidity. For example, T<sub>H17</sub> cells were shown to span a continuum of states, from higher expression of a program associated with pathogenic effect to one characteristic of regulatory cells, with distinct regulators for each program (6). Single-cell RNA-seq also provides a way for lineage tracing by capturing the sequence of the T cell receptor transcript (10, 44, 45).

Profiling of chromatin organization, especially histone marks, across different T<sub>H</sub> cell types highlighted how epigenetic memory maintains cell state stably, while remaining sufficiently malleable to allow for plasticity (Fig. 3). Although signature cytokines often have a chromatin pattern congruent with strict cell type definitions, chromatin at other key signature genes of one lineage is not always repressed in other T<sub>H</sub> lineages, offering a possible basis for future plasticity. Indeed, chromatin marks and accessibility can change even for signature cytokines or TFs after stimulation (46). Conversely, DNA demethylation and stable chromatin organization, with contribution from chromatin regulators and long noncoding RNAs, play an important role in stability. For instance, demethylation of specific regulatory elements in a CpG island in the locus of Foxp3, a key regulator of T<sub>reg</sub> cells, helps stabilize the cells’ identity, further reinforced by a transcriptional positive feedback loop (47). The ability of chromatin organization to function as a malleable memory device is reflected by the preponderance of DNA variants associated with autoimmune disease that map to enhancer regions in T<sub>H</sub> cells (46).

Finally, RNA and TF ChIP-seq profiles, combined with genetic perturbations, have helped shed light on the intricate intracellular circuits controlling these processes in T<sub>reg</sub> (49) and inflammatory T<sub>H</sub>17 cells (50, 51). For example, in T<sub>H17</sub> cells, they revealed a “yin-yang” network of TFs, with two densely connected self-reinforcing, but mutually antagonistic, modules: A larger module promotes the T<sub>H17</sub> cell fate and suppresses alternative fates, and a smaller module has an opposite function (Fig. 3). The dense, interconnected positive module provides stability, as has also been proposed for other T<sub>H</sub> lineages (42, 49). The smaller negative module could promote alternative plastic fates.

**Malleable cell states mirror tissue location**

Immune cells sense, adapt, respond to, and affect their environment in the context of the tissue (Fig. 1D). Tissue-resident immune cells, sometimes lifelong sessile inhabitants, play critical
roles in homeostasis and pathology, well beyond responses to pathogens. Tissue-resident macrophages perform unique functions as “accessory cell types” (1) that serve “client” primary cells defining the respective tissue. For example (1, 23), alveolar macrophages are critical for surfactant homeostasis in the lungs, microglia are essential for synaptic pruning in the brain, osteoclasts are critical for the dynamic balance of bone, and splenic red-pulp macrophages help manage heme and iron from aging red blood cells. Tissue-resident Tregs (5) have been identified in visceral adipose tissue (VAT) (52, 53), the intestine (54), muscle (55), and lung (56), with roles from metabolic homeostasis to tissue repair and regeneration.

Genomic analysis has played a critical role in identifying tissue-resident immune cells, characterizing their unique features, determining their tissue-specific functions, and inferring the principles and mechanisms by which they adapt to the diversity of tissues in the body and their changing local conditions.

RNA profiling has identified the level at which immune cells of a single “type” vary given their tissue of residence (Fig. 3). In addition to a core set of macrophage-associated genes, tissue-resident macrophages (18, 21, 40, 57) express distinct gene modules in each tissue type. For example, brain-resident microglia (which are deposited prenatally), develop in lockstep with the rest of the central nervous system during brain development and are susceptible to environmental signals prenatally (58). T_{regs} isolated from different tissues have shown similar distinctions (52–55). The profiles and derived gene signatures then become the fingerprint of the cell’s identity, and—when coupled

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Fig. 3. Key features, hypotheses, and principles revealed by genomic studies of immune-environment interactions. (A) Three key genomic tools used to analyze transcription and epigenetic mechanisms that participate in immune cell responses. For each tool (left), shown are the main features it characterizes, testable hypotheses derived by computational analysis, and current emerging principles and mechanisms from such studies. (B) Example of key principles and mechanisms derived by genomic study of immune response to the environment. (Left) Consecutive transcriptional waves (top) are driven by sequential activation of regulators in a negative feedback loop (bottom) such that a regulator active at an earlier time point (yellow) activates (arrow) another regulator at a subsequent time point (green), which in turn represses (blunt arrow) the earlier regulator. (Second from left) Dense interconnected circuits and regulator reuse and foreshadowing in differentiation. Each transient and terminal cell state in differentiation is associated with a distinct dense interconnected circuit of regulators (circularly arranged nodes and arrows) with the same regulators (colored nodes) "reused" in multiple cell types and lineage, and regulators active at a later step in differentiation already affecting the cell (e.g., through chromatin structure) at earlier stages. (Third from left) Cell fate plasticity. Two densely interconnected modules of regulators (red and blue circularly arranged nodes, respectively) control a cell at a given fate 1 (top) (e.g., T_{α,17} cell), positively (arrow) and negatively (blunt arrow), respectively, with the inverse effect on alternative fates (bottom) and a mutually inhibitory effect (double blunt arrow in middle) on each other. (Right) Tissue-specific epigenomes. The epigenome (DNA, red; nucleosomes, gray; bound TFs, crescents) of tissue resident cells (right, e.g., macrophages) shares some common characteristics that are also present in their progenitors (left) (e.g., open chromatin region in center) but may also include features (e.g., enhancers and bound TFs) that are unique to the tissue of residence.
to transfer, chimera, or lineage-tracing experiments—
can establish whether a cell is stably resident in a
tissue (32). It is possible that other immune
lineages may also follow such principles; single-
cell profiling of entire tissues (9, 10) will help de-
terminate this.

Individual genes expressed in these tissue-
specific modules—including TFs, cytokines, che-
molines, and receptors—provide critical starting
points to determine the cells’ functions (e.g., lipid
metabolism in T_{reg} in VAT (59), regulatory mecha-
nisms (e.g., Gata6 in peritoneal macrophages (57)),
and interaction with other tissue cells (e.g.,
T_{reg}–adipocyte interaction through IL-10 (53)).

The exquisite tunability reflected by these pro-
grams led in turn to the exploration of how they
are indeed shared across all tissues but
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Perspective: Toward a tissue circuit

The cellular environment is interwoven into a
single integrated whole in tissues, bringing together
diverse cell types—epithelial, immune, neural,
stromal, and more—as they differentiate and

Dissecting how cells interact to maintain

Addressing these questions requires the ability to

Analysis at a whole-tissue level should provide

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