(Schroeder et al., 2008) is highly expressed in inner supporting cells and the calcium transient activated by ATP generates a substantial chloride efflux current in these cells. Surprisingly, the burst firing of inner hair cells is not due to the direct activation of purinergic receptors on hair cells but is instead due to the ATP-triggered calcium signals and chloride efflux in the glial-like supporting cells. Burst firing in hair cells is lost when expression of TMEM16a channels is reduced or eliminated in the inner supporting cells. The clue to the mechanism came from the similarities with observations between fluid secretion from epithelial cells and the shape change of the inner supporting cells. ATP triggers crenation, the term for osmosis-induced cell shrinkage, in inner supporting cells due to the efflux of water associated with the substantial chloride efflux observed after TMEM16a activation. In exocrine epithelia of various organs the efflux of chloride via TMEM16a (Huang et al., 2012) causes the concurrent efflux of K+ and water to maintain ionic and osmotic gradients (Frizzell and Hanrahan, 2012). Wang et al. show that the efflux of K^+ from inner supporting cells is sufficient to increase extracellular to approximately 12 mM (Wang et al., 2015). This change in external [K^+] is both required and sufficient for inner supporting cells to trigger calcium spike bursting in inner hair cells.

In the central nervous system, astrocytes are the glial cells that keep extracellular [K^+] within a very narrow range around 3 mM (Kofuji and Newman, 2004). Increases of several mM are generated by neuronal activity and seizures are characterized by external [K^+] up to 13-15 mM. K^+ efflux could occur from astrocytes due to spatial buffering by glial networks when K^+ diffuses through astrocytes from regions of high to low extracellular [K^+] (Kofuji and Newman, 2004). Astrocytes also have a GABA-activated Cl^- current, first proposed by Bormann and Kettenmann (Bormann and Kettenmann, 1988) to generate K^+ efflux to depolarize neurons. The discovery of the mechanism underlying K^+ efflux from inner supporting cells and the profound impact on hair cell calcium bursting will certainly lead to investigations on the impact of Cl^- and associated K^+ effluxes from astrocytes in regulating neuronal circuit excitability in the developing central nervous system.

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A Metabolic Switch for Th17 Pathogenicity

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T helper 17 (Th17) cells are critical for host defense but can also drive autoimmunity. This divergent behavior is explored by Gaublomme et al. and Wang et al., who identify inflammation-associated genes by measuring gene expression in nearly 1,000 individual Th17 cells and show that *CD5L* affects the expression of pro-inflammatory genes by altering lipid synthesis.

T helper (Th) cells are workhorses of adaptive immunity, which as their name implies, help other immune cells respond appropriately to pathogens. The Th paradigm began with two lineages: Th1 cells that respond to intracellular pathogens and Th2 cells that respond to extracellular parasites. A third lineage, Th17 cells, was identified more recently (Harrington et al., 2006; Stockinger and Veldhoen, 2007; Toh and Miossec, 2007). As various groups began to map the mechanisms that specified their development, the heterogeneity of Th17 cells began to be appreciated (Ghoreschi et al., 2010). While originally the role of Th17 cells in autoimmunity was emphasized, it has become clear that non-pathogenic Th17 cells in the gut are controlled by the microbiome and are critical for intestinal barrier function (Littman and Rudensky, 2010). However, the molecular mechanisms underlying these divergent behaviors remain relatively poorly understood.

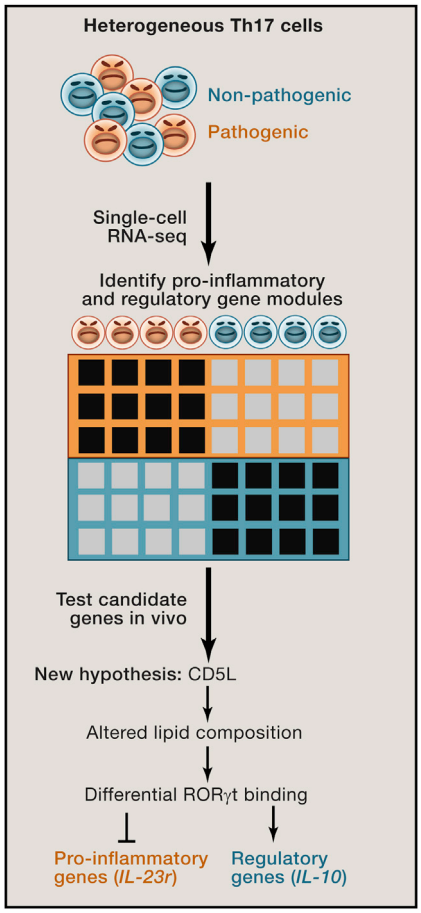

Figure 1. Measuring Gene Expression in Single Th17 Cells Reveals a Metabolic Switch for Pathogenicity

Th17 cells are functionally heterogeneous, with some critical for host defense (non-pathogenic) and others driving autoimmunity (pathogenic). Gaublomme et al. (2015) characterize the genetic basis of this heterogeneity by measuring gene expression in individual Th17 cells. They find two distinct modules of genes that correlate with pathogenicity: a pro-inflammatory module that correlates positively with inflammatory genes and a regulatory module that correlates negatively with these genes. Wang et al. (2015) show that *CD5L* is expressed in non-pathogenic cells, where it negatively regulates pro-inflammatory genes. CD5L acts as a metabolic switch that alters the balance of lipid saturation and affects the availability of ligands for RORγt, the master transcription factor of Th17 cells. Although the mechanism is not yet clear, the effect of CD5L on lipid metabolism reduces RORγt binding at the pathogenic *IL-23r* and *IL-17* genes and increases binding at the protective *IL-10* gene.

Understanding this issue is not simply an intriguing intellectual exercise but has direct clinical relevance with respect to the divergent consequences of targeting IL-17 in various diseases ranging from psoriasis to inflammatory bowel disease.

The heterogeneity of Th17 function also points to the fundamental question of how to define a cell type. The operating definition for Th17 cells is IL-17 production, but this criterion does not distinguish pathogenic from non-pathogenic cells. Cellular heterogeneity is an issue that immunologists and cell biologists in general have confronted for years. Flow cytometry revolutionized immunology by allowing high-throughput counting and purifying of subpopulations based on cell-surface markers. New methods to measure genes expressed in single cells are a powerful complement to flow cytometry, enabling transcript-based sorting of cellular heterogeneity.

In this issue, single-cell measurements of gene expression are used in conjunction with traditional genetics and immunology to identify regulators of Th17 cells. Gaublomme et al. (2015) measure the genes expressed in nearly 1,000 individual Th17 cells generated in vitro or purified from an in vivo model of multiple sclerosis to relate their expression pattern to Th17 pathogenesis. This comprehensive survey of ~1,000 Th17 "individuals" identifies two distinct modules of genes: a pro-inflammatory module whose expression levels positively correlated with known pro-inflammatory genes and a regulatory module whose expression levels negatively correlate with these genes. Furthermore, these new measurements reveal a set of genes, including potential regulators, that otherwise ranked poorly in previous bulk population studies. Among newly identified potential regulators, Wang et al. (2015) show that *CD5L* acts as a metabolic switch to link the metabolic state of Th17 cells to its master regulator RORγt (Littman and Rudensky, 2010). Together, these studies convincingly demonstrate the power of single-cell genomics to characterize cellular heterogeneity and unveil key molecules contributing to differences in cell state and function (Figure 1).

It is interesting to note that, in Wang et al.'s analysis, *CD5L* has a counterintuitive pattern of expression. Although its expression level correlates with the pro-inflammatory module, the gene was expressed only in non-pathogenic cells. This pattern suggests that it is a negative regulator. The authors confirm the role of CD5L in vivo by showing that *CD5L* expression level correlates with the severity of experimental autoimmune encephalomyelitis symptoms. The most fascinating aspect of this story comes next, when they ask how *CD5L* works. Previous work has shown that CD5L is secreted by macrophages and endocytosed by adipocytes, where it directly binds fatty acid synthase (Kurokawa et al., 2010). This led the authors to hypothesize that *CD5L* affects lipid biosynthesis in Th17 cells. In fact, the authors show that *CD5L* alters the balance of polyunsaturated and saturated fatty acids, specifically affecting two metabolic genes, *cyp51* and *sc4mol*, which synthesize endogenous ligands for RORγt—the master transcription factor in Th17 cells. *CD5L*-deficient mice have increased RORγt binding at the *IL-17* and *IL-23r* loci (both pro-inflammatory genes) and decreased binding at *IL-10* (a regulatory gene). They also show that altering the balance of lipid saturation mimics the effect of CD5L deficiency.

This new link from lipid metabolism through transcription factor binding to Th17 pathogenicity is tantalizing but also raises the obvious question of specificity. How can the availability of lipid ligands for RORγt selectively increase its binding in some parts of the genome and reduce its binding in others? Other proteins are likely involved, as transcription factor binding is often a combinatorial affair, but the precise mechanism of specificity is an exciting question to explore further. The authors clearly demonstrate the importance of *CD5L* in controlling Th17 function, but how is *CD5L* itself controlled? A puzzle in the new findings suggests that this mechanism is complicated: Stat3 promotes *CD5L* expression, and IL-23 promotes *Stat3* expression, yet somehow IL-23 suppresses *CD5L*. Elucidating how *CD5L* expression is regulated is another open avenue of work.

In summary, Gaublomme and Wang demonstrate the power of single-cell measurements of gene expression to map the genetic basis of Th17 functional heterogeneity. Measuring the genes expressed in single cells is becoming cheaper and of higher throughput and is already proving valuable for surveying cell diversity in complex tissues like the

retina (Macosko et al., 2015). In addition to measuring transcriptional output, new methods have also been developed to characterize transcriptional regulation by measuring methylated DNA, accessible chromatin, modified histones, and chromatin conformation in single cells (Schwartzman and Tanay, 2015). Of course, technical hurdles remain. Measuring genes expressed in single cells is noisy, and existing methods suffer from low sensitivity. Methods to characterize chromatin in single cells are even less mature and face harder limits on the dynamic range of their measurements. However, if history is a guide, these methods will be improved rapidly and together form a suite of tools to systematically discover new cell types and map the genetic control of their phenotype and function.

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Interleukin-18: The Bouncer at the Mucosal Bar

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The fidelity of the intestinal barrier is critical to maintaining a healthy relationship between the immune system and the microbiota. Levy et al. and Nowarski et al. reveal how microbiota-derived metabolites modulate the activation of the inflammasome to influence the expression of the cytokine IL-18, intestinal barrier function, and intestinal inflammation.

The mucosal immune system has a complex task, as it must be vigilant to pathogens while maintaining cordial relations with the relatively benign commensal microbiota. To complicate matters, inflammation in the intestine can allow the outgrowth of aggressive members of the microbiota, blurring the lines between "pathogens" and "commensals" and contributing to autoinflammatory conditions such as inflammatory bowel disease (Dalal and Chang, 2014). A primary mechanism of immune homeostasis in the gut is to limit the interaction with the microbiota via the physical barrier made of the intestinal epithelial cells (IECs), anti-microbial proteins, and the mucus, produced by goblet cells (Hooper and Macpherson, 2010). The inflammasome, a macromolecular structure that supports the post-translational production of the cytokines IL-1β and IL-18, plays a critical role in supporting the intestinal barrier. As a result, mice deficient in inflammasome function and IL-18 production develop an invasive dysbiotic microbiota that exacerbates pathology in mouse models of chemically induced colitis (Elinav et al., 2013). Two papers in this issue of *Cell* now better elucidate how the inflammasome and microbiota interact via sensing of metabolites to induce IL-18 expression, modulate intestinal barrier function, and intestinal inflammation (Levy et al., 2015; Nowarski et al., 2015).

Previous studies on mice deficient in key components of the inflammasome have indicated that this structure may support goblet cell secretion and therefore intestinal barrier function, independent of the production of IL-18 (Wlodarska et al., 2014). Levy et al. (2015) now extend these findings to show that, at steady state, signals from the microbiota are necessary for inflammasome activation, IL-18 production, and the expression of certain anti-microbial proteins (AMPs). Critically, one of these AMPs, Ang4, is sufficient to restore microbial diversity, providing an explanation of how IL-18 supports the intestinal barrier and why abrogation of IL-18 may lead to commensal dysbiosis (see Figure 1). In contrast, during instances of acute inflammation, IL-18 may exacerbate disease. Using a series of genetic tools to parse the role of IL-18 during chemically induced colitis, Nowarski et al. (2015) show that IL-18 signaling specifically