Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Biotechnology

Metabolic regulation of epigenetic remodeling in immune cells

Emily C Britt[1,2], Steven V John[1,2], Jason W Locasale[3] and Jing Fan[1,2]

Immune cells are capable of sensing various signals in the microenvironment and turning on specific immune functions in response. The appropriate transition of immune cells into diverse functional states, which is crucial for immunity, involves complex and well-regulated changes in transcriptional program. Accumulating evidence shows that epigenetic remodeling plays a central role in mediating the transcriptional program for immune cell activation and immunological memory. Concurrently, immune cells undergo significant metabolic reprogramming during immune response. Here we review recent studies that demonstrate shifts in metabolic state can orchestrate immune cell functions through its impact on epigenetic remodeling, and the microenvironment can exert its influence on immune cells through the metabolic regulation of epigenetics. We also discuss the systems biology approaches that enabled these discoveries.

Addresses
[1] Morgridge Institute for Research, Madison, WI 53715, USA
[2] Department of Nutritional Sciences, University of Wisconsin-Madison, Madison, WI 53706, USA
[3] Department of Pharmacology and Cancer Biology, Duke University School of Medicine, Durham, NC 27705, USA

Corresponding author: Fan, Jing (jfan@morgridge.org)

Current Opinion in Biotechnology 2020, **63**:xx-yy

This review comes from a themed issue on **Systems biology**

Edited by **Marco Vanoni** and **Pasquale Palumbo**

https://doi.org/10.1016/j.copbio.2019.12.008

Introduction

It has been well recognized that metabolism has a significant impact on epigenetic regulation via a variety of molecular mechanisms: Key metabolites can act as substrates, inhibitors, or activators of the covalent modifications of histones, DNA and RNA, and some metabolic enzymes can directly participate in epigenetic remodeling. These molecular mechanisms have been intensively discussed in recent reviews [1–4], and therefore are not discussed in detail here.

This connection between metabolism and epigenetics allows cells to integrate information about metabolic state, which reflects nutrient availability, environmental condition, and cellular health, into the cell fate decision. The regulatory role of metabolism on epigenetics has wide implications in physiological transitions, such as development [5,6], as well as pathological conditions, such as cancer [7,8]. Immune responses are tightly controlled processes coupled with massive reprogramming in both metabolism and epigenetics. High-throughput technologies have allowed quantitative characterization of the diverse epigenetic landscape and metabolic modes in immune cells in various states, and opened up active research on the impact of metabolism on epigenetics and the function of immune cells. This metabolism-epigenetics regulatory axis allows precise and dynamic control of immunity, and dysregulation of this process can contribute to many diseases. Here we discuss recent discoveries in this area.

Epigenetics in the activation and memory of immune cells

Innate immune cells, such as macrophages and neutrophils, can rapidly respond to molecular signals, including cytokines and pathogen-associated molecular patterns (PAMPs), and become activated accordingly. Epigenetic remodeling, here referred to as dynamics in gene regulation, for example due to changes in chromatin status, plays a crucial role in the activation process. Modification of histones creates new accessible chromatin sites and allows binding of specific transcription machinery, which permits the expression of specific genes, many functioning in pathogen killing, inflammation, or tissue remodeling. Recent research has revealed that innate immune cells can also form immunological memory, where epigenetic remodeling is an important underlying mechanism as well. In macrophages following lipopolysaccharide (LPS) stimulation, many genes become activated and have increased histone acetylation and H3K4 trimethylation. Some genes retain the H3K4 trimethylation but lose the acetylation mark quickly, and remain inaccessible upon secondary stimulation; while other genes retain the acetylation on their promoter, and can be turned on even faster upon secondary stimulation [9]. Using ChipIP-sequencing analysis, it has been revealed that the modifications of latent enhancers (enhancers that can only be bound by transcription factors after stimulation) are also an important mechanism for transcriptional memory in macrophages. Latent enhancers do not have

activating histone marks such as H3K27 acetylation and H3K4 monomethylation in unstimulated macrophages, but acquire these marks after stimulation. After the first stimulation, these marks can persist, allowing faster and amplified responses upon re-stimulation [10,11]. The immunological memory in macrophages is not only mediated by acquiring new epigenetic marks, but also by the decrease of some epigenetic marks in comparison to unstimulated macrophages. For instance, upon LPS stimulation, the repressive H3K9 dimethylation is decreased in macrophages through an ATF7-dependent mechanism [12]. This chromatin remodeling de-represses a set of genes and can be maintained for a long time.

The control of innate immunity through epigenetic remodeling is not limited to immune cells with a long lifetime such as macrophages. Neutrophils are highly abundant, short-lived leukocytes that function as the front line of immunity. While the epigenetic remodeling in neutrophils is relatively less understood, several recent papers have demonstrated its role in neutrophil activation, recruitment, and function. For instance, an increase of H3K4 trimethylation and H3K27 and H4 acetylation upon stimulation promotes binding of transcription machinery and IL-6 production in neutrophils [13]. Histone acetylation promotes migration, phagocytosis and neutrophil extracellular trap formation, and loss of deacetylase HDAC11 leads to hyperacetylation and increased neutrophil activation, making animals more susceptible to LPS-induced sepsis [14,15]. Precise control of histone acetylation is a mechanism for controlling neutrophil activation. The transcription factor PU.1 prevents neutrophil overactivation by recruiting a histone deacetylase and inhibiting the accessibility of enhancers [16]. Dynamic chromatin remodeling has also been demonstrated in natural killer (NK) cells. Using ATAC-seq and RNA-seq, Lau *et al.* revealed that during early viral infection, NK cells show a change in chromatin accessibility that coordinates with transcriptional changes, and that memory NK cells have a distinct epigenetic landscape from naïve NK cells [17].

Similar to innate immune cells, epigenetic regulation is necessary in T cell development, activation, and maintenance of subtypes. Many of these aspects have been thoroughly reviewed recently [18•]. High-throughput

Table 1

Cutting edge technologies and approaches to study metabolism-epigenetic interactions in immune cells

Approach	Description	Examples for application
ChIP-sequencing	Analyzes protein-DNA interaction. ChIP-seq is a valuable tool to identify the enrichment of specific histone marks or transcription factor binding in the genome.	ChIP-seq analysis revealed increased H3K4me3 and H3K27Ac at promoters of metabolic genes induced by β-glucan training in monocytes [57]. In neutrophils, ChIP was used to identify genomic sites bound by important transcription factor PU.1 [16].
ATAC-seq	Analyzes genome-wide chromatin accessibility.	ATAC-seq was used to identify chromatin regions with increased accessibility caused by acetate supplementation in T cells grown in glucose restricted environment [43••].
Bisulfite sequencing	Analyzes DNA methylation pattern by converting cytosine to thymidine, which methylated cytosine is resistant to.	Whole genome bisulfide sequencing identified genomic regions that are differentially methylated in different T cell populations, depending on their specific tissue environment [40].
RNA-seq	High-throughput method to quantify RNA levels. mRNA-seq is widely used to determine the changes in gene expression during immune response.	Using RNA-seq, Novakovic *et al.* characterized the clusters of genes whose dynamic expression patterns differ in response to LPS exposure or β-glucan exposure, and revealed the relationship between exposure-dependent expression pattern and epigenetic remodeling by integrating RNA-seq with ChIP-seq results [58].
Metabolomics	High-throughput method to quantify metabolite levels. Can be achieved by NMR or mass spectrometry.	Jha *et al.* used metabolomics to systematically characterize how the metabolic profile of naïve macrophages and macrophages in M1 or M2 activation state differ, and identified metabolic modules important in macrophage polarization by integrating metabolomics with RNA-seq data using a network analysis method [21].
Isotopic tracing	By following the incorporation of labeled substrate into downstream metabolite or epigenetic modifications, it can be determined how metabolism is rewired during immune response and how various substrate or pathways contribute to epigenetic remodeling.	Using methionine labeling, Sinclair *et al.* probed changes in methionine uptake, metabolism, and methionine-driven methylation in T cells in response to TCR [24•]. Using acetate labeling, Qiu *et al.* investigated the contribution of acetate in histone acetylation, and how it is dependent on ACSS activity in T cells [43••].

sequencing technologies have allowed systematic characterization of epigenetic remodeling during these processes, and contributed to the discovery of gene clusters controlled by specific epigenetic mechanisms (Table 1). For instance, using ATAC-seq, an approach to analyze open chromatin, it was revealed that chromatin remodeling in T cell development occurs in waves, and the lineage-determining transcription factor T cell factor 1 (TCF-1) controls T cell identity through its ability to create new open chromatin [19]. RNA-sequencing and ChIP-seq revealed that permissive chromatin configurations, which are installed after initial stimulation, are partially maintained in memory T cells, allowing for rapid responses upon reactivation [20].

Metabolism orchestrates immune cell function via epigenetic mechanisms

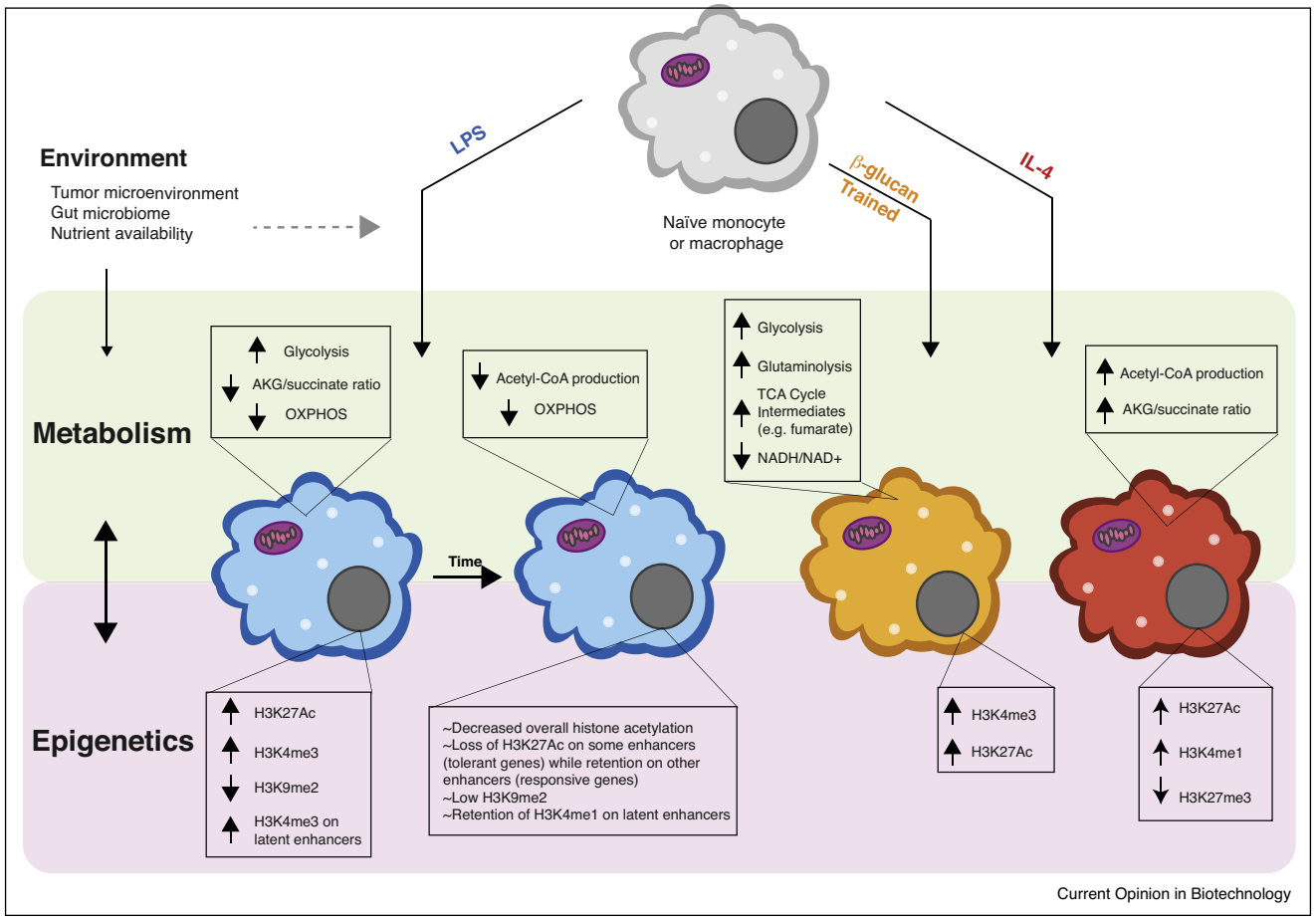
As the epigenetic landscape undergoes significant remodeling during immune response, cellular metabolism also exhibits dynamic reprogramming specific to the activation signal [21–23]. Metabolism provides substrates for the highly active epigenetic modifications. Therefore the levels of epigenetic-modulating metabolites can affect functions in immune cells.

During T cell activation, the demand of methyl donor *S*-adenosyl methionine (SAM) increases as cells increase the methylation rate of DNA, RNA and histones. Using metabolomics, proteomics, and isotopic labeling approaches, Sinclair *et al.* found that the main regulation point controlling the flux for SAM supply is the import of extracellular methionine, the main substrate for SAM, in CD4+ T cells. Rapid upregulation of the methionine transporter is required for sustaining methylation activity and allowing full activation of T cells [24•]. In macrophages, high SAM/*S*-adenosyl homocysteine (SAH) ratio promotes LPS-stimulated production of pro-inflammatory cytokine IL-1β, by supporting H3K36 trimethylation. Inhibiting SAM synthesis or SAH degradation alters cytokine profile [25]. Similar to methylation, the acetylation of histones and other proteins during immune response is under control of the level of its main substrate, acetyl-CoA. Upon activation, CD4+ T cells increase aerobic glycolysis to maintain high acetyl-CoA levels, which promotes histone acetylation and interferon-γ (IFNγ) expression. Deletion of lactate dehydrogenase A reduces aerobic glycolysis, decreases IFNγ production in activated T cells, and protects mice against autoinflammatory disease [26]. In addition to glucose, CD8+ memory T cells have been shown to take up acetate from the serum to increase the acetyl-CoA pool and promote acetylation upon infection [27]. In macrophages, IL-4 stimulation promotes glucose driven acetyl-CoA production by regulating ATP-citrate lyase. This leads to increased histone acetylation and induction of M2 genes [28]. Conversely, M1 activation, induced by LPS stimulation, suppresses mitochondrial metabolism, and at the same time decreases overall histone acetylation [29]. This effect is particularly strong after prolonged LPS stimulation, which contributes to LPS tolerance by limiting the histone acetylation and the activation of inflammatory genes (Figure 1) [30•]. The limitation of acetyl-CoA availability in M1 macrophages can occur through modulation of glycerol phosphate shuttle and inhibition of pyruvate dehydrogenase [30•,31].

In addition to the installation of histone and DNA modifications, the epigenetic landscape is also controlled by the reactions removing these modifications. Particularly, demethylation of histones and DNA can be catalyzed by α-ketoglutarate (AKG)-dependent demethylases. Several metabolites that are structurally similar to AKG, including succinate, fumarate, and 2-hydroxy-glutarate (2HG) can competitively inhibit demethylation. Therefore, the ratio of AKG to these inhibitory metabolites modulates methylation levels. These metabolites are intermediates in the TCA cycle or directly derived from the TCA cycle, and a lot of these compounds are largely derived from glutamine. This allows TCA cycle activity and glutamine metabolism to regulate immune cells through modulation of demethylases. In macrophages, the AKG/succinate ratio is increased upon IL-4 stimulation (M2) but decreased upon LPS stimulation (M1). Liu *et al.* showed that manipulating AKG/succinate ratio altered macrophage polarization in a dose-dependent manner, with increasing AKG/succinate ratio promoting M2 gene expression while decreasing AKG/succinate ratio strengthening M1 phenotypes [32••]. Memory in macrophages and monocytes is also regulated by TCA metabolism. Arts *et al.* revealed that induction of glucose and glutamine metabolism is crucial in trained immunity. Treating monocytes with fumarate was sufficient to induce an epigenetic program, including increased H3K4 trimethylation at the promoters of pro-inflammatory cytokines, similar to monocytes trained with β-glucan (Figure 1) [33•]. If glycolysis or glutaminolysis is inhibited, monocytes are unable to induce trimethylation of histones at the promoters of pro-inflammatory cytokines IL-6 and TNFα, which is an important part of BCG-induced, trained immunity [34]. And glutaminolysis can modulate LPS-induced tolerance in macrophages [32••]. Similarly, the ratio of AKG and metabolites that inhibit demethylation regulates the fate of T cells. In CD8+ T cells, 2HG accumulates in response to T cell receptor triggering. Increase of 2HG, particularly S-2HG, leads to an increase of global H3K27 trimethylation and decrease of its dimethylation, and alters T cell differentiation and proliferation [35]. In CD4+ T cells, transamination activity controls 2HG production. Increased 2HG level causes hypermethylation of *Foxp3* gene, and shifts the balance between Th17 and induced Treg cells [36•].

Besides the mechanisms discussed above where metabolites directly modulate epigenetic modifications by

Figure 1



Coordinated changes in metabolism and epigenetics in macrophages/monocytes during polarization by different stimulation signals or formation of immunological memory. Abbreviations: LPS, lipopolysaccharide; IL-4, interleukin-4; AKG, α-ketoglutarate; OXPHOS, oxidative phosphorylation; H3K27Ac, acetylation of histone H3K27; H3K4me3, trimethylation of histone H3K4; H3K9me2, dimethylation of histone H3K9; H3K4me1 monomethylation of H3K4.

acting as substrates or inhibitors, metabolism can impact epigenetics through indirect mechanisms involving the production of metabolites with signaling properties. For instance, the accumulation of mevalonate, an intermediate in cholesterol metabolism, can induce trained immunity in monocytes through activation of IGF1 receptor and subsequent histone modifications [37]. The metabolism and release of other signaling molecules, such as eicosanoids, can also activate corresponding receptors and trigger downstream epigenetic remodeling [38].

The metabolism-epigenetic axis allows microenvironment to shape immune cells

Microenvironment has a significant impact on the epigenetic landscape of immune cells. For instance, studies that leverage multiple systematic sequencing approaches, including whole genome bisulfide sequencing (WGBS), ChIP-seq, ATAC-seq and RNA-seq, to compare the immune cells isolated from various tissues have demonstrated the epigenetic diversity of macrophages and T cells is dependent on the specific tissue they reside [39,40]. At the same time, metabolism of immune cells is also tissue-specific [41]. Two types of mechanisms link the specific metabolic environment with the epigenetic changes in immune cells: (1) nutrient availability and environmental condition shape the metabolism in immune cells, which in turn affect their epigenetics; (2) metabolites produced by other cells in the microenvironment can affect the epigenetics in immune cells.

In the tumor microenvironment, low availability of glucose, glutamine and oxygen is common, due to insufficient blood perfusion and high metabolic activity of cancer cells. Glucose restriction dampens effector T cell functions. One mechanism is through altering histone methylation [42]. Another mechanism is that low glucose availability restricts acetyl-CoA level, which is required for histone acetylation. Acetate supplementation can

rescue effector CD8+ T cell functions in a low glucose environment by enhancing histone acetylation and chromatin accessibility [43••]. In addition to glucose restriction, glutamine deprivation can promote the generation of Treg cells from a naïve CD4+ T cell population by limiting AKG availability, which is needed for DNA demethylation [44]. Hypoxia can also alter T cell phenotypes and anti-tumor capacity by influencing methylation [35]. In the gut microenvironment, the microbiome can regulate immune cells by producing regulatory metabolites, particularly short chain fatty acids. For instance, butyrate regulates local macrophage function by inhibiting histone deacetylases, and promotes tolerance to bacteria in such environments [45]. In B cells, short chain fatty acids regulate gene expression by increasing histone acetylation, which is likely due to both inhibition of histone deacetylation and increase in acetyl-CoA level [46]. The regulatory connection between metabolism and epigenetics also allows diet to influence immune function. A Western diet can trigger inflammation, and induce epigenetic reprogramming and trained immunity in granulocyte-monocyte progenitor cells [47].

Discussion and perspective

Increasing studies demonstrate that metabolism plays a crucial role in regulating immune cell functions. As discussed here, modulating the epigenetic landscape is an important mechanism for such regulation. Other mechanisms, including metabolism impacting signaling pathways, metabolic enzymes directly regulating transcription, and metabolism influencing organelle function, also allow metabolism to regulate immunity. These are discussed in several recent reviews [48•,49]. As a result, metabolic intervention is emerging as a promising strategy to modulate immunity [50].

While here we focused on immune cells, and mainly discussed how chromatin modification, in the form of acetylation and methylation, can be regulated by metabolism, the connection between metabolism and epigenetics is more ubiquitous. Other less characterized modifications can link metabolism with epigenetics as well. For instance, Zhang *et al.* demonstrated that a novel histone modification, lysine lactylation, is regulated by intracellular lactate level. In macrophages, lactate production from glycolysis induced by bacteria exposure causes increased histone lactylation, which then induces the expression of a set of genes during the late phase of M1 polarization [51•]. Metabolic regulation of epigenetics also widely occurs in other types of cells besides immune cells. Altered metabolism is a hallmark in cancer. The important role of metabolic alteration in impacting cancer epigenome and disease progression has been shown in many studies over the last decade, and has been thoroughly reviewed [52,3,53]. For instance, accumulation of 2HG, the first identified oncometabolite produced in large quantity in glioma harboring mutant IDH, causes DNA and histone hypermethylation. Metabolism influences epigenetics during development and cell fate decision through a similar mechanism as well [3,5]. It is also worth noting that this metabolism-epigenetics connection is not limited to a mammalian system. For example, in yeast, acetyl-CoA level acts as a signal for carbon metabolism and can promote proliferation through histone acetylation [54].

It has been proposed and demonstrated in other biological systems that epigenetic modifications can act as a sink and/or reservoir for metabolites [55]. It is also likely that some of the metabolic rewiring observed in immune cells is caused by the increased metabolic demand due to highly active epigenetic remodeling during immune response. For instance, inflammatory macrophages become dependent on NAD^+ salvage pathway due to high NAD^+ consumption for poly-ADP-Ribosylation [56]. It is also possible that demands for metabolic substrates, such as acetate, would drive epigenetic changes in immune cells. Quantitative analysis of overall epigenetic remodeling flux during immune response, and how that compares to the production flux of required metabolite substrates, is still largely missing.

Many important open questions remain to be answered in this area: Which of the metabolism-influenced epigenetic changes are reversible whereas which persist for a longer period? What molecular mechanisms allow metabolism to regulate the chromatin of a specific set of genes at a specific time window? Furthermore, the crosstalk between metabolism and epigenetics during immune response is bidirectional: the metabolic alterations can be both a consequence and a driver for epigenetic remodeling. For instance, glycolysis enzymes are among the most upregulated genes by histone modifications in β-glucan trained macrophages [57]. Therefore, feed-forward and feed-back regulation may drive the dynamic transitions in both metabolism and epigenetics. Future studies working toward dynamic understanding of the metabolism-epigenetic regulation network would provide insights into these questions. Development of methods that integrate high-throughput analysis of epigenetic landscape and metabolism is needed to explore these intriguing interactions.

Author contribution statement

All authors reviewed the literature and determined the framework and content of this review. E.C.B and J.F wrote most of the manuscript, S.V.J and J.W.L edited the manuscript.

Conflict of interest statement

Nothing declared.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Kaelin WG, McKnight SL: **Influence of metabolism on epigenetics and disease**. *Cell* 2013, **153**:56-69.

2. Keating ST, El-Osta A: **Epigenetics and metabolism**. *Circ Res* 2015, **116**:715-736.

3. Reid MA, Dai Z, Locasale JW: **The impact of cellular metabolism on chromatin dynamics and epigenetics**. *Nat Cell Biol* 2017, **19**:1298-1306.

4. Fan J, Krautkramer KA, Feldman JL, Denu JM: **Metabolic regulation of histone post-translational modifications**. *ACS Chem Biol* 2015, **10**:95-108.

5. Cantone I, Fisher AG: **Epigenetic programming and reprogramming during development**. *Nat Struct Mol Biol* 2013, **20**:282-289.

6. John RM, Rougeulle C: **Developmental epigenetics: phenotype and the flexible epigenome**. *Front Cell Dev Biol* 2018, **6**:130.

7. Flavahan WA, Gaskell E, Bernstein BE: **Epigenetic plasticity and the hallmarks of cancer**. *Science* 2017, **357**:eaal2380.

8. Dawson MA, Kouzarides T: **Cancer epigenetics: from mechanism to therapy**. *Cell* 2012, **150**:12-27.

9. Foster SL, Hargreaves DC, Medzhitov R: **Gene-specific control of inflammation by TLR-induced chromatin modifications**. *Nature* 2007, **447**:nature05836.

10. Kaikkonen MU, Spann NJ, Heinz S, Romanoski CE, Allison KA, Stender JD, Chun HB, Tough DF, Prinjha RK, Benner C *et al.*: **Remodeling of the enhancer landscape during macrophage activation is coupled to enhancer transcription**. *Mol Cell* 2013, **51**:310-325.

11. Ostuni R, Piccolo V, Barozzi I, Polletti S, Termanini A, Bonifacio S, Curina A, Prosperini E, Ghisletti S, Natoli G: **Latent enhancers activated by stimulation in differentiated cells**. *Cell* 2013, **152**:157-171.

12. Yoshida K, Maekawa T, Zhu Y, Renard-Guillet C, Chatton B, Inoue K, Uchiyama T, Ishibashi K, Yamada T, Ohno N *et al.*: **The transcription factor ATF7 mediates lipopolysaccharide-induced epigenetic changes in macrophages involved in innate immunological memory**. *Nat Immunol* 2015, **16**:1034-1043.

13. Zimmermann M, Aguilera FB, Castellucci M, Rossato M, Costa S, Lunardi C, Ostuni R, Girolomoni G, Natoli G, Bazzoni F *et al.*: **Chromatin remodelling and autocrine TNFα are required for optimal interleukin-6 expression in activated human neutrophils**. *Nat Commun* 2015, **6**:6061.

14. Sahakian E, Chen J, Powers JJ, Chen X, Maharaj K, Deng SL, Achille AN, Lienlaf M, Wang HW, Cheng F *et al.*: **Essential role for histone deacetylase 11 (HDAC11) in neutrophil biology**. *J Leukoc Biol* 2017, **102**:475-486.

15. Hamam HJ, Khan MA, Palaniyar N, Hamam HJ, Khan MA, Palaniyar N: **Histone acetylation promotes neutrophil extracellular trap formation**. *Biomolecules* 2019, **9**:32.

16. Fischer J, Walter C, Tönges A, Aleth H, Jordão MJC, Leddin M, Gröning V, Erdmann T, Lenz G, Roth J *et al.*: **Safeguard function of PU.1 shapes the inflammatory epigenome of neutrophils**. *Nat Immunol* 2019, **20**:546-558.

17. Lau CM, Adams NM, Geary CD, Weizman OE, Rapp M, Pritykin Y, Leslie CS, Sun JC: **Epigenetic control of innate and adaptive immune memory**. *Nat Immunol* 2018, **19**:963-972.

18. • Schmidl C, Delacher M, Huehn J, Feuerer M: **Epigenetic mechanisms regulating T-cell responses**. *J Allergy Clin Immunol* 2018, **142**:728-743.

This review summarizes epigenetic mechanisms, including DNA methylation and histone modifications, in regulating the differentiation, proliferation and functions in T cells.

19. Johnson JL, Georgakilas G, Petrovic J, Kurachi M, Cai S, Harly C, Pear WS, Bhandoola A, Wherry EJ, Vahedi G: **Lineage-determining transcription factor TCF-1 initiates the epigenetic identity of T cells**. *Immunity* 2018, **48**:243-257.e10.

20. Barski A, Cuddapah S, Kartashov AV, Liu C, Imamichi H, Yang W, Peng W, Lane HC, Zhao K: **Rapid recall ability of memory T cells is encoded in their epigenome**. *Sci Rep* 2017, **7**:39785.

21. Jha AK, Huang SC-C, Sergushichev A, Lampropoulou V, Ivanova Y, Loginicheva E, Chmielewski K, Stewart KM, Ashall J, Everts B *et al.*: **Network integration of parallel metabolic and transcriptional data reveals metabolic modules that regulate macrophage polarization**. *Immunity* 2015, **42**:419-430.

22. Klein Geltink RI, Kyle RL, Pearce EL: **Unraveling the complex interplay between T cell metabolism and function**. *Annu Rev Immunol* 2018, **36**:461-488.

23. Ryan DG, O-Neill LAJ: **Krebs cycle rewired for macrophage and dendritic cell effector functions**. *FEBS Lett* 2017, **591**:2992-3006.

24. • Sinclair LV, Howden AJ, Brenes A, Spinelli L, Hukelmann JL, Macintyre AN, Liu X, Thomson S, Taylor PM, Rathmell JC *et al.*: **Antigen receptor control of methionine metabolism in T cells**. *eLife* 2019, **8**.

This work integrated metabolomics, proteomics, and isotopic tracing to investigate methylation during T cell response, and identified the import of methionine as the rate-limiting factor in generating methyl groups for protein synthesis and methylation of DNA, RNA and histone in T cells.

25. Yu W, Wang Z, Zhang K, Chi Z, Xu T, Jiang D, Chen S, Li W, Yang X, Zhang X *et al.*: **One-carbon metabolism supports S-adenosylmethionine and histone methylation to drive inflammatory macrophages**. *Mol Cell* 2019, **75**:1147-1160.e5.

26. Peng M, Yin N, Chhangawala S, Xu K, Leslie CS, Li MO: **Aerobic glycolysis promotes T helper 1 cell differentiation through an epigenetic mechanism**. *Science* 2016, **354**:481-484.

27. Balmer ML, Ma EH, Bantug GR, Grählert J, Pfister S, Glatter T, Jauch A, Dimeloe S, Slack E, Dehio P *et al.*: **Memory CD8+ T cells require increased concentrations of acetate induced by stress for optimal function**. *Immunity* 2016, **44**:1312-1324.

28. Covarrubias AJ, Aksoylar HI, Yu J, Snyder NW, Worth AJ, Iyer SS, Wang J, Ben-Sahra I, Byles V, Polynne-Stapornkul T *et al.*: **Akt-mTORC1 signaling regulates Acly to integrate metabolic input to control of macrophage activation**. *eLife* 2016, **5**.

29. Tong W-H, Maio N, Zhang D-L, Palmieri EM, Ollivierre H, Ghosh MC, McVicar DW, Rouault TA: **TLR-activated repression of Fe-S cluster biogenesis drives a metabolic shift and alters histone and tubulin acetylation**. *Blood Adv* 2018, **2**:1146-1156.

30. • Langston PK, Nambu A, Jung J, Shibata M, Aksoylar HI, Lei J, Xu P, Doan MT, Jiang H, MacArthur MR *et al.*: **Glycerol phosphate shuttle enzyme GPD2 regulates macrophage inflammatory responses**. *Nat Immunol* 2019, **20**:1186-1195 http://dx.doi.org/10.1038/s41590-019-0453-7.

This study demonstrated that glycerol phosphate shuttle affects inflammatory responses in macrophages by regulating acetyl-coA production and histone acetylation.

31. Seim GL, Britt EC, John SV, Yeo FJ, Johnson AR, Eisenstein RS, Pagliarini DJ, Fan J: **Two-stage metabolic remodelling in macrophages in response to lipopolysaccharide and interferon-γ stimulation**. *Nat Metab* 2019, **1**:731-742.

32. •• Liu P-S, Wang H, Li X, Chao T, Teav T, Christen S, Di Conza G, Cheng W-C, Chou C-H, Vavakova M *et al.*: **α-ketoglutarate orchestrates macrophage activation through metabolic and epigenetic reprogramming**. *Nat Immunol* 2017, **18**:985-994.

This paper showed that the ratio of α-ketoglutarate to succinate is crucial in orchestrating the polarization of macrophages and mediating endotoxin tolerance.

33. • Arts RJW, Novakovic B, ter Horst R, Carvalho A, Bekkering S, Lachmandas E, Rodrigues F, Silvestre R, Cheng S-C, Wang S-Y *et al.*: **Glutaminolysis and fumarate accumulation integrate**

immunometabolic and epigenetic programs in trained immunity. *Cell Metab* 2016, **24**:807-819.
This work identified-specific metabolic activities are required for development of trained immunity in monocytes. Particularly, fumarate has an important role in trained immunity through epigenetic mechanisms.

34. Arts RJW, Carvalho A, La Rocca C, Palma C, Rodrigues F, Silvestre R, Kleinnijenhuis J, Lachmandas E, Gonçalves LG, Belinha A *et al.*: **Immunometabolic pathways in BCG-induced trained immunity**. *Cell Rep* 2016, **17**:2562-2571.

35. Tyrakis PA, Palazon A, Macias D, Lee KL, Phan AT, Veliça P, You J, Chia GS, Sim J, Doedens A *et al.*: **S-2-hydroxyglutarate regulates CD8+ T-lymphocyte fate**. *Nature* 2016, **540**:236-241.

36. • Xu T, Stewart KM, Wang X, Liu K, Xie M, Ryu JK, Li K, Ma T, Wang H, Ni L *et al.*: **Metabolic control of TH17 and induced Treg cell balance by an epigenetic mechanism**. *Nature* 2017, **548**:228-233.
This paper showed that production of metabolite 2-hydroxyglutate can shift the balance between different CD4+ T cells by affecting methylation of important genes.

37. Bekkering S, Arts RJW, Novakovic B, Kourtzelis I, van der Heijden CDCC, Li Y, Popa CD, Ter Horst R, van Tuijl J, Netea-Maier RT *et al.*: **Metabolic induction of trained immunity through the mevalonate pathway**. *Cell* 2018, **172**:135-146.e9.

38. Fu P, Ebenezer DL, Ha AW, Suryadevara V, Harijith A, Natarajan V: **Nuclear lipid mediators: role of nuclear sphingolipids and sphingosine-1-phosphate signaling in epigenetic regulation of inflammation and gene expression**. *J Cell Biochem* 2018, **119**:6337-6353.

39. Lavin Y, Winter D, Blecher-Gonen R, David E, Keren-Shaul H, Merad M, Jung S, Amit I: **Tissue-resident macrophage enhancer landscapes are shaped by the local microenvironment**. *Cell* 2014, **159**:1312-1326.

40. Delacher M, Imbusch CD, Weichenhan D, Breiling A, Hotz-Wagenblatt A, Träger U, Hofer A-C, Kägebein D, Wang Q, Frauhammer F *et al.*: **Genome-wide DNA-methylation landscape defines specialization of regulatory T cells in tissues**. *Nat Immunol* 2017, **18**:1160-1172.

41. Caputa G, Castoldi A, Pearce EJ: **Metabolic adaptations of tissue-resident immune cells**. *Nat Immunol* 2019, **20**:793-801.

42. Zhao E, Maj T, Kryczek I, Li W, Wu K, Zhao L, Wei S, Crespo J, Wan S, Vatan L *et al.*: **Cancer mediates effector T cell dysfunction by targeting microRNAs and EZH2 via glycolysis restriction**. *Nat Immunol* 2016, **17**:95-103.

43. •• Qiu J, Villa M, Sanin DE, Buck MD, O?"Sullivan D, Ching R, Matsushita M, Grzes KM, Winkler F, Chang C-H *et al.*: **Acetate promotes T cell effector function during glucose restriction**. *Cell Rep* 2019, **27**:2063-2074.e5.
This work showed acetate supplementation can alter T cell function in glucose restricted environment by providing substrate for acetylation. It exemplified the influence of microenvironment on immune cell function via metabolic regulation of epigenetics.

44. Klysz D, Tai X, Robert PA, Craveiro M, Cretenet G, Oburoglu L, Mongellaz C, Floess S, Fritz V, Matias MI *et al.*: **Glutamine-dependent α-ketoglutarate production regulates the balance between T helper 1 cell and regulatory T cell generation**. *Sci Signal* 2015, **8**:ra97.

45. Chang PV, Hao L, Offermanns S, Medzhitov R: **The microbial metabolite butyrate regulates intestinal macrophage function via histone deacetylase inhibition**. *Proc Natl Acad Sci U S A* 2014, **111**:2247-2252.

46. Kim M, Qie Y, Park J, Kim CH: **Gut microbial metabolites fuel host antibody responses**. *Cell Host Microbe* 2016, **20**:202-214.

47. Christ A, Günther P, Lauterbach MAR, Duewell P, Biswas D, Pelka K, Scholz CJ, Oosting M, Haendler K, Ba?xler K *et al.*: **Western diet triggers NLRP3-dependent innate immune reprogramming**. *Cell* 2018, **172**:162-175.e14.

48. • Buck MD, Sowell RT, Kaech SM, Pearce EL: **Metabolic instruction of immunity**. *Cell* 2017, **169**:570-586.
This review highlights recent discoveries on how metabolism and metabolic environment affect immune cell functions.

49. Rambold AS, Pearce EL: **Mitochondrial dynamics at the interface of immune cell metabolism and function**. *Trends Immunol* 2018, **39**:6-18.

50. Mulder WJM, Ochando J, Joosten LAB, Fayad ZA, Netea MG: **Therapeutic targeting of trained immunity**. *Nat Rev Drug Discov* 2019, **18**:553-566.

51. • Zhang D, Tang Z, Huang H, Zhou G, Cui C, Weng Y, Liu W, Kim S, Lee S, Perez-Neut M *et al.*: **Metabolic regulation of gene expression by histone lactylation**. *Nature* 2019, **574**:575-580.
This work identified novel histone lysine lactylation, and demonstrated that it can stimulate gene expression and is under the regulation of lactate level.

52. Kinnaird A, Zhao S, Wellen KE, Michelakis ED: **Metabolic control of epigenetics in cancer**. *Nat Rev Cancer* 2016, **16**:694-707.

53. Miranda-Gonçalves V, Lameirinhas A, Henrique R, Jerónimo C: **Metabolism and epigenetic interplay in cancer: regulation and putative therapeutic targets**. *Front Genet* 2018, **9**.

54. Cai L, Sutter BM, Li B, Tu BP: **Acetyl-CoA induces cell growth and proliferation by promoting the acetylation of histones at growth genes**. *Mol Cell* 2011, **42**:426-437.

55. Ye C, Tu BP: **Sink into the epigenome: histones as repositories that influence cellular metabolism**. *Trends Endocrinol Metab* 2018, **29**:626-637.

56. Cameron AM, Castoldi A, Sanin DE, Flachsmann LJ, Field CS, Puleston DJ, Kyle RL, Patterson AE, Hässler F, Buescher JM *et al.*: **Inflammatory macrophage dependence on NAD+ salvage is a consequence of reactive oxygen species-mediated DNA damage**. *Nat Immunol* 2019, **20**:420-432.

57. Cheng S-C, Quintin J, Cramer RA, Shepardson KM, Saeed S, Kumar V, Netea MG: **mTOR- and HIF-1a-mediated aerobic glycolysis as metabolic basis for trained immunity**. *Science (80-)* 2013, **342** 1242454.

58. Novakovic B *et al.*: **β-Glucan Reverses the Epigenetic State of LPS-Induced Immunological Tolerance**. *Cell* 2016, **167(5)**:1354-1368.e14.