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Advances in understanding molecular regulation of innate immune memory

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Abstract

The epigenetic and functional reprogramming of immune genes during induction of trained immunity is accompanied by the metabolic rewiring of cellular state. This memory is induced in the hematopoietic niche and propagated to daughter cells, generating epigenetically and metabolically reprogrammed innate immune cells that are greatly enhanced in their capacity to resolve inflammation. In particular, these cells show accumulation of H3K4me3 and H3K27Ac epigenetic marks on multiple immune gene promoters and associated enhancers. However, the mechanism governing how these epigenetic marks accumulate at discrete immune gene loci has been poorly understood, until now. Here, we discuss some recent advances in the regulation of trained immunity, with a particular focus on the mechanistic role of a novel class of long non-coding RNAs in the establishment of epigenetic marks on trained immune gene promoters.

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Introduction

A fundamental element in the survival of organisms confronted with continuous exposure to potentially pathogenic microorganisms is the presence of a strong and dynamic defense system. Two different levels of immune response are recognized: innate and adaptive. The first one is regarded as the first line of defense against the entry of microbes and the rapid elimination of those that have already penetrated the organism before they cause infection. This response provides nonspecific protection and is based on the recognition of evolutionarily preserved structures found in the pathogens known as pathogen-associated molecular patterns that act as ligands of the pattern recognition receptors, expressed by the cells of the innate immune system. Innate immune cells with the capacity to act as antigen-presenting cells such as macrophages and dendritic cells process and present microbial peptides to the adaptive branch of the immune system, providing antigen-specific protection against reinfection. Approximately 400 million years ago, during the Cambrian, vertebrates developed mechanisms for generate diversity that depend on recombination of the VDJ elements and the presence of *Rag1* and *Rag2* genes. The genomic recombination provided by these systems allows the evolution of the immune system of vertebrates and the emergence of genes related with the adaptive immune system of mammals. Based on these mechanisms, vertebrates have developed the capacity to 'remember' the antigen or pathogen with which they have had contact, entailing a large evolutionary advantage.

Innate immune memory in vertebrates: trained immunity

It is increasingly evident that the innate immune responses are far more sophisticated than the previously

held view in which they are primitive responses that are triggered each time in the same fashion. An increasing amount of evidence shows that stimulation of the cells triggers a functional reprogramming in cells of the innate immune system, such as monocytes and natural killer (NK) cells, facilitating a faster and enhanced response to secondary challenges with the same or other stimuli [1]. This innate immunological memory in response to nonspecific stimuli has been termed 'trained immunity' [2]. Long-term epigenetic and metabolic reprogramming of monocytes and macrophages is necessary to provide increased responsiveness against systemic heterologous infections in vertebrates [1] and can also be induced in alveolar macrophages by a respiratory viral infection, thanks to the local release of IFNγ by effector CD8+ lymphocytes in mice [2]. Exposure of human NK cells to *Candida albicans*-induced metabolic reprogramming in these cells increasing their production of perforin [3]. The potential of induction of innate immune memory is not restricted to microbial ligands. Endogenous molecules, such as oxLDL, glucose or uric acid in high concentrations are also able to trigger these mechanisms and lead to the development of chronic inflammatory responses such as those seen in patients with diabetes, atherosclerosis, gout, or cancer [4–6]. Trained immunity has also been linked with physiological processes such as human pregnancy, where NK cells support vascular sprouting and improved placentation [7]. Novel research also suggests that the features of trained immunity are not strictly restricted to immune cells but also to other cells such as fibroblasts or stromal cells [8], epithelial cells [9] and that the long-term consequences of trained immunity are transmitted through durable modifications in the epigenetic landscape of the cells of the hematopoietic niche [10,11]. These nonimmune cells express receptors for several inflammatory mediators, which enables them to adjust to the inflammatory milieu [3–5]. Moreover, stromal and epithelial cells are also equipped with the receptors to sense whether the epithelial barrier is breached and, in turn, actively recruit the immune cells [6]. Trained immunity also provides an explanation for the heterologous effects of vaccines: the capacity of some vaccines to provide immune protection against infections other than the specific target, with reduced all-cause mortality [12,13].

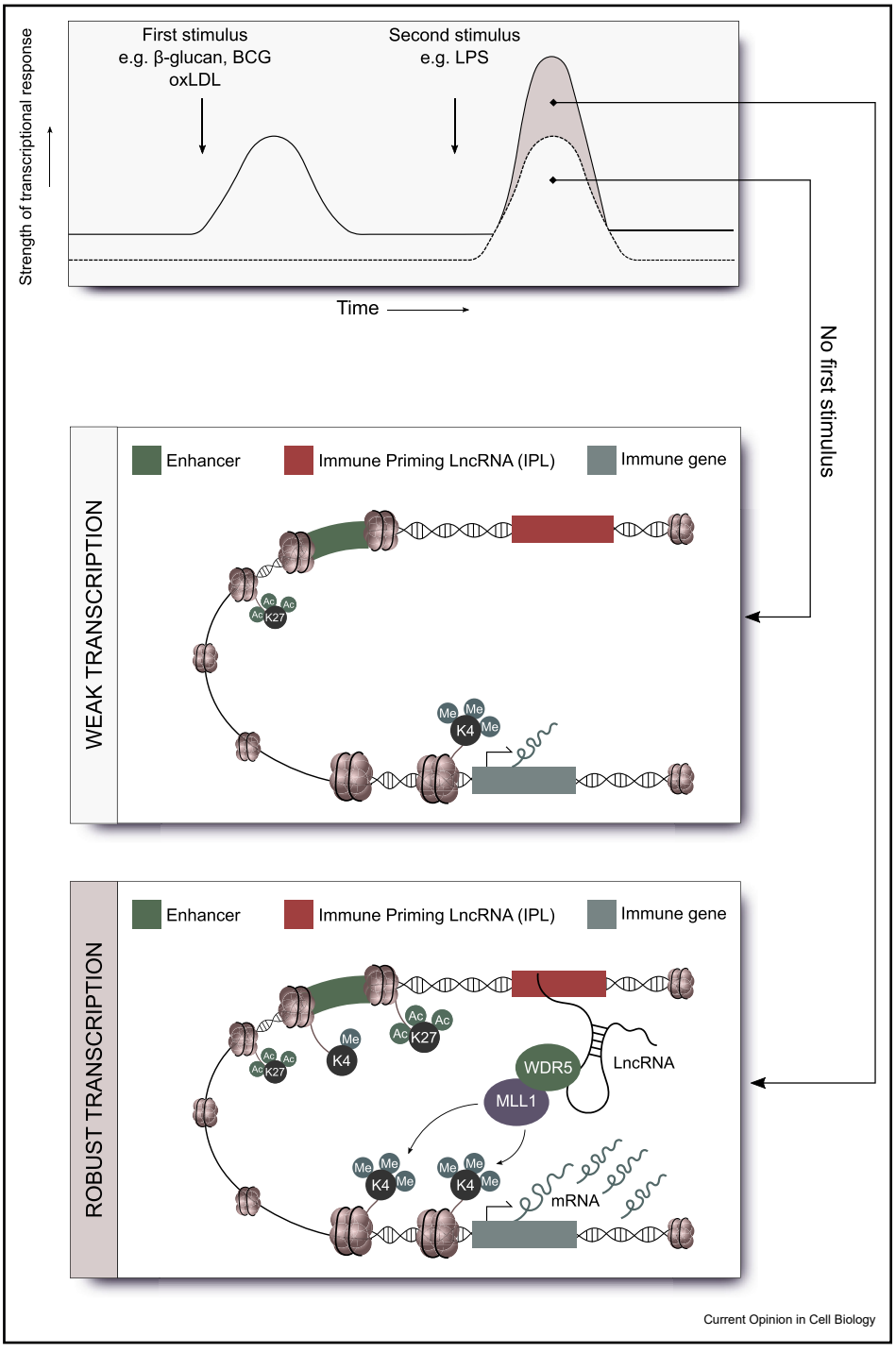
The immune pathways leading to epigenetic changes

The induction of trained immunity relies on the activation of intracellular elements, such as the Akt-mTOR–HIF–1α pathway, what leads to the recruitment of transcriptional regulators of immune processes, such as NF-kB and nuclear factor of activated T cells (NFAT) [7]. During primary stimulation, the activation of gene transcription is accompanied by the deposition of specific histone acetylation and methylation marks, such as H3K4me3 or H3K27ac, gene regulatory elements that regulate the expression of proinflammatory factors. This process increases the accessibility of the DNA to the transcriptional machinery and regulatory elements, promoting and facilitating enhanced transcription [14] (Figure 1). Challenge of monocytes with β-glucan also leads to the enrichment of acetylation of histone 3 lysine 27 (H3K27ac), monomethylation of histone 3 lysine 4 (H3K4me1), trimethylation of histone 3 lysine 4 (H3K4me3), and increased DNase I accessibility across specific loci of monocytes. The deposition of H3K27ac marks is often paralleled by that of H3K4me1. Therefore, even if a considerable portion of the H3K27ac marks are lost during cell differentiation, the remaining H3K4me1 marks heritably maintain an open conformation of the chromatin and as a consequence remain sensitive to cleavage by DNase I [15]. The enrichment of H3K4me3 at the promoters of loci encoding proinflammatory genes plays a central role in different experimental models of innate immune memory, such as β-glucan stimulation or the induction of trained immunity by oxLDL in human cells [16]. In line with this, H3K4me3 enrichment at the promoters of genes encoding TNFα and IL-6 is necessary for the increased responsiveness observed after the vaccination of healthy volunteers with bacille Calmette-Guérin (BCG) [17]. In contrast, the treatment with *E. coli*-derived lipopolysaccharide delays the establishment of all these chromatin marks in human cells [14]. This failure to deposit active histone marks at promoters of proinflammatory genes is behind the lack of responsiveness to secondary stimulation (also known as immune tolerance) in response to a secondary challenge with lipopolysaccharide [14]. Epigenetic changes underlying chronic proinflammatory gene expression in situations of hyperglycemia have been related with a persistent, long-lasting H3K4me1 in proinflammatory genes [18]. Although these studies have focused on commonly studied histone marks, such as H3K27Ac and H3K4me3, there are a large number of different histone marks that may act in a combinatorial manner to activate or repress transcription. Clearly, future studies are required to investigate how trained immunity influences the deposition of other chromatin modifications (e.g. H3K9 and H3K14 acetylation on active enhancers and/or promoters, and methylation of H3K36 in transcribed gene bodies). This insight is necessary to provide additional insight into how the longevity of epigenetic memory is established.

The integration of metabolic pathways and epigenetic changes

The changes observed in the epigenetic landscape are concurrent with the metabolic reprogramming of the cells. The stimulation of innate immune cells with microbial or endogenous ligands involved in the induction of

Figure 1



The molecular basis of trained immunity is underpinned by the epigenetic reprogramming of immune genes. Innate immune cells (such as circulating monocytes and macrophages) exposed to stimuli (including β-glucan, bacille Calmette-Guérin -BCG- and oxLDL) are epigenetically reprogrammed. As a consequence of this, upon exposure to a secondary stimulus, immune genes are more robustly transcribed. This process is regulated by a novel class of lncRNAs, called immune priming lncRNAs (IPL), which are upregulated by the initial stimulus. IPLs directly interact with WDR5, to direct MLL1 proximal to immune genes, facilitating the deposition of H3K4me3 at the promoters of immune genes. In this way, immune genes are more robustly transcribed upon secondary infection/stimulus in trained immune cells. lncRNAs, long non-coding RNAs, MLL1, mixed lineage leukemia protein 1; WDR5, WD repeat-containing protein 5.

innate immune memory mechanisms alters the genetic landscape of metabolic regulators. In this sense, different genes involved in glycolysis, tricarboxylic acid (TCA) cycle, glutaminolysis, and the cholesterol pathway are quickly upregulated after stimulation, causing changes in the levels of intracellular metabolites in mice and human [8–10]. Variations in the levels of intracellular metabolites derived from these pathways alter the functionality of enzymes responsible of 'writing', 'erasing' or 'reading' histone and DNA modifications that alter the epigenetic landscape of the innate immune cells. In this regard, acetyl-CoA derived from glycolysis and glutaminolysis is able to act as a donor of acetyl groups for acetylation of histones [19]. Accumulation of citrate facilitates the production of soluble factors that promote inflammation such as derivatives of arachidonic acid or nitric oxide [20]. Increased levels of itaconate after microbial stimulation is related with a decrease in the deposition of epigenetic marks in monocytes, leading to the development of immune tolerance [21]. The presence of α-ketoglutarate is fundamental for the activity of the ten-eleven translocation (TET) proteins, leading to the elimination of DNA repressor marks [22]. These TET proteins are involved in the remodeling of the DNA methylation landscape of cells from the hematopoietic niche in the bone marrow and thus are crucial for the long-term reprogramming of systemic innate immune responses after β-glucan or BCG challenge in mice [10,11]. Although this relationship is yet to be shown in humans, this strongly suggests that the TET proteins will have a significant impact on innate immune cell reprogramming.

Histone lysine demethylases of the JmjC and JmjD family also need α-ketoglutarate as a cofactor to induce demethylation [23]. S-adenosyl methionine, formed from adenosine and methionine through the enzyme methionine adenosyltransferase, is a common cosubstrate involved in the transfer of methyl groups and necessary for the actions of histone and DNA methyltransferases (DNMTs) [24]. Succinate induces the stabilization of the transcription factor HIF1α, which triggers an intracellular pathway that leads to the accumulation of H3K4me3 and H3K27ac in the promoter regions and regulatory areas of proinflammatory genes through a pathway that also involves the activation of mTOR and Akt [25,26]. For its part, fumarate antagonizes the hydroxylation reaction necessary for HIF1α degradation, contributing to the stabilization of this factor and inhibition of the activity of the KDM5 family of histone demethylases [27]. This enhances the long-term reprogramming of the epigenetic landscape of monocytes, skewing them toward a trained phenotype that favors an increased responsiveness after secondary stimulation. The epigenetic profiles of monocytes and macrophages challenged by different ligands that induce innate immune memory, such as β-glucan, BCG, oxLDL, or uric acid, show close similarities among each other at a mechanistic level [6,10,11,15].

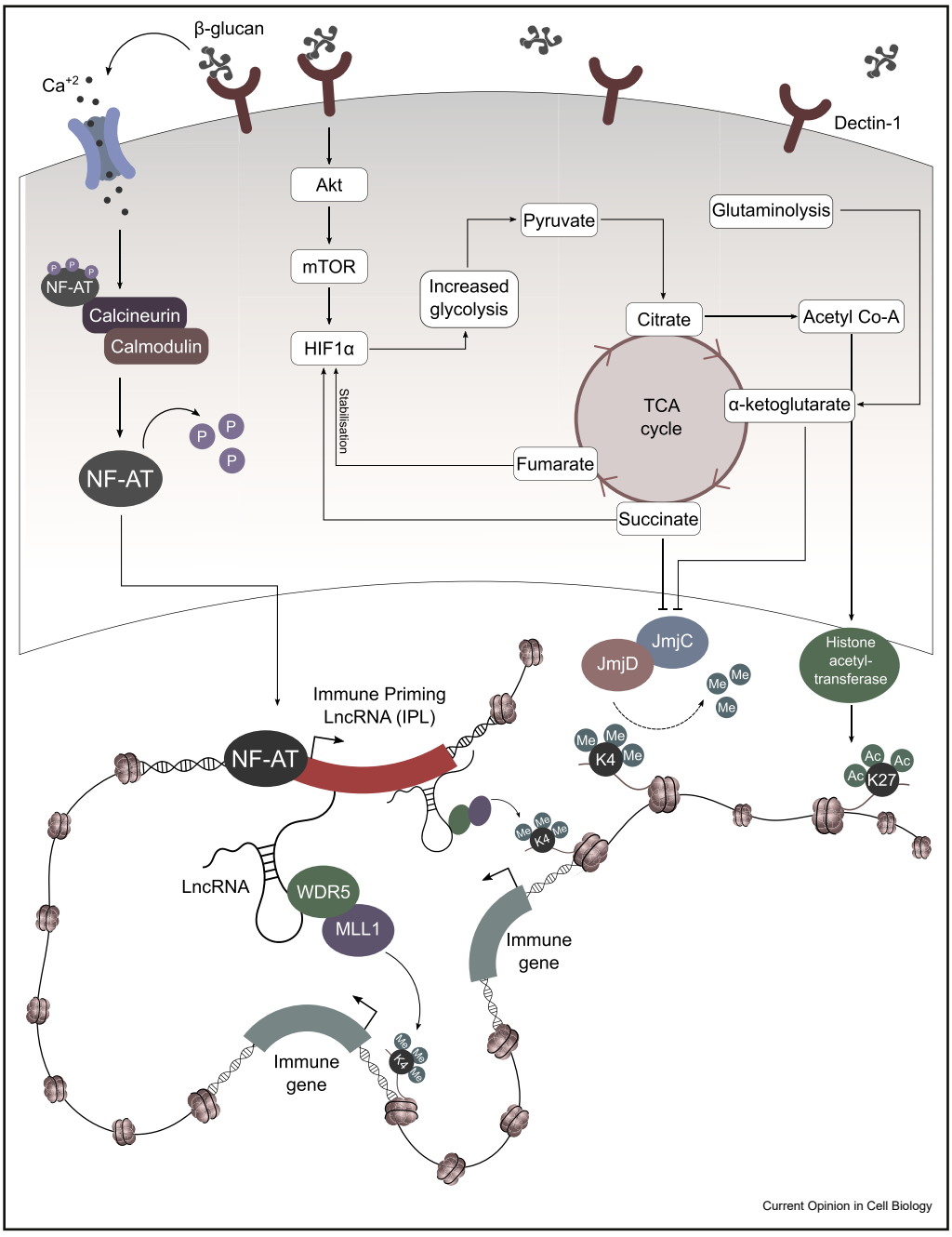
The role of lncRNAs and 3D nuclear architecture in trained immunity

Although it is clear that immune genes and associated enhancers are epigenetically reprogrammed during trained immunity, it is poorly understood how epigenetic remodeling enzymes (such as mixed lineage leukemia protein 1 [MLL1]) are discretely targeted to trained immune genes and enhancer elements. Clearly, gene regulatory mechanisms facilitate the precise targeting of these chromatin remodelers, at the correct spatiotemporal location and in a context-specific manner.

By regulating immune gene transcription, the noncoding portion of the genome has been shown to play an integral regulatory role in the regulation of inflammatory processes [28]. It appears that a significant portion of the genome is transcribed into a highly diverse family of RNAs, that range in size from >200 nt to more than 10 Kbp in length. Many of these so-called long noncoding RNAs (lncRNAs) are key modulators of gene regulation that act at various stages of the transcriptional program to either amplify or repress gene activity [29]. lncRNAs are distinct from protein coding RNAs, in that they may act in *cis* to regulate genes in their surrounding genomic neighborhood. As a consequence, very few copies of lncRNAs (even 1 or 2 copies per cell) may significantly influence gene regulation. However, owing to technical challenges in their detection and functional characterization, it can be difficult to determine whether lncRNA transcripts are indeed functional and not simply the by-product of transcriptional noise [30]. Therefore, despite the fact that thousands of lncRNAs have been identified, the complete molecular function of only a few lncRNAs has been described. Several well-designed studies have revealed that lncRNAs can serve as functional transcripts that play an important role in the development of disease states, including cancer, infectious disease, and inflammation [31]. These studies reveal that lncRNAs can act in *cis* or *trans* via diverse mechanisms that includes acting as scaffolds, decoys, and recruiters of chromatin remodelers (extensively reviewed in [29,31]). For example, careful mechanistic analysis revealed that lincRNA-Cox2 interacts with hnRNPA2/B1 and hnRNP-A/B to repress a large number of immune genes [32]. LncRNAs such as NeST and HOXA distal transcript antisense RNA (HOTTIP) have been convincingly shown to interact with WD repeat-containing protein 5 (WDR5) and direct MLL1 to target genes in *cis*, allowing the deposition of H3K4me3 at the promoters of IFNG and the HOXA genes, respectively [28,33].

The folding of chromatin in three dimensions has a significant impact on gene regulation [34,35]. High-throughput chromosome conformation capture and associated techniques (chromatin interaction analysis by

Figure 2



An overview of the interplay between metabolism and epigenetics during trained immunity. β-glucan/dectin-1 signaling activates calcium-dependent NFAT signaling, to induce the transcription of the IPLs resulting in the H3K4me3 epigenetic reprogramming of immune gene promoters. Together with enhancer elements, IPLs are able to access target genes via 3D chromosomal looping. Concurrently, there is the activation of mTOR-HIF1α signaling, which alters the activity of different intracellular pathways. As a consequence, there is an increase in the supply of metabolites and cofactors that are essential to consolidate the epigenetic changes that are causal to the trained immunity phenotype. IPLs, immune gene priming lncRNAs.

paired-end tag sequencing (Hi-C, and so on) have revealed that chromatin is folded into DNA loops, which are spatially segregated into topologically associating domains [34]. Chromosomal looping within topologically associating domains has been shown to bring distally located lncRNAs and their protein partners adjacent to target genes to regulate their transcriptional activation. For example, HOTTIP has been shown to use 3D chromatin topology to direct the WDR5/MLL1 complex proximal to the *HOXA* genes [33].

As these factors are central to epigenetic regulation, we recently explored the contribution of lncRNAs and 3D nuclear architecture in the regulation of trained immunity in human cells [11]. Using a novel bioinformatic pipeline, we identified several lncRNAs which we named immune gene priming lncRNAs (IPLs) [36]. One candidate IPL, which we have named upstream master lncRNA of the inflammatory chemokine locus, engaged in chromosomal contacts with the promoters of the ELR + CXCL chemokines (IL-8, CXCL1, CXCL2, and CXCL3). Using loss- and gain-of-function experiments, we demonstrated that upstream master lncRNA of the inflammatory chemokine locus uses 3D nuclear topology to direct the WDR5/MLL1 complex across the CXCL chemokine promoters. This enables the H3K4me3 epigenetic priming of their promoters before transcriptional activation. Importantly, this mechanism was shared with other key trained immune genes, such as IL-6 and IL1β. At the transcriptional level, exposure of monocytes to β-glucan resulted in the an NFAT-mediated increase in the expression of IPLs, which in turn resulted in the epigenetic reprogramming of innate immune genes (Figure 2). The promoters of IPLs contain multiple transcription factor binding motifs, for example, STAT, AP-1, and RELA. This suggests that divergent stimuli that activate distinct signal transduction cascades may converge on the activation of IPLs. This may explain how different training agents (e.g. β-glucan, oxLDL, BCG) that activate different receptors are able to induce IPL expression and epigenetically reprogram immune genes.

Enhancer RNAs and trained immunity

Enhancers may also be transcribed into lncRNAs called enhancer RNAs (eRNAs) which are typically expressed in a cell-specific manner. In several instances, the transcripts that arise from enhancers have been shown to play a significant role in the regulation of chromosomal looping and tissue-specific target gene transcription [37]. For example, in separate studies, eRNAs have been shown to interact with components of the mediator complex or Yin Yang 1 to regulate chromosomal contacts between target genes and enhancers [37,38]. Recently, eRNAs have been shown to interact with p300 and cyclic adenosine monophosphate response element–binding protein, which are two highly conserved proteins that possess histone acetyltransferase activity [39]. The study revealed that eRNAs bind to p300 and cyclic adenosine monophosphate response element–binding protein, which in turn stimulates catalytic histone acetyltransferase activity and results in transcriptional activation. Therefore, it is reasonable to speculate that by regulating histone acetylation and looping at key enhancers, eRNAs may play an integral role in trained immune responses.

Conclusions and future perspectives

In vivo studies have shown the persistence of epigenetic BCG-induced memory in mice up to 20 weeks [12] after vaccination and up to a month in humans vaccinated with BCG [13]. At present, it remains unknown whether this enhanced protection after several years is also accompanied by epigenetic memory. Recent studies have demonstrated that epigenetic memory is induced in the hematopoietic niche, which is then propagated to daughter cells [10,11]. In this way, long-term innate immune memory may be established. What is less clear is the contribution of lncRNAs and enhancers to the generation and maintenance of long-term memory. We speculate that hematopoietic stem cells (HSCs) may be trained in an IPL-dependent manner, similar to what occurs within circulating monocytes trained with β-glucan [40]. The pathways that upregulate the IPLs, may also lead to the transcription of enhancer elements, which would in turn increase their accessibility and acetylation. These epigenetic changes would then be transmitted from HSCs and progenitors to the mature monocytes and macrophages. Therefore, deciphering the function of these noncoding elements will be critical to elucidate the exact mechanism of how trained immunity is established.

There is strong evidence that other 'classes' of lncRNA may regulate aspects of immune memory. By methylating cytosines (m5C) in CpG-rich sequences, DNMTs are able to induce transcriptional repression. Several studies reveal that all three major DNMTs can bind and be regulated by lncRNAs [14]. Therefore, it is tempting to speculate that there may be a class of immune 'suppressing' lncRNAs that may directly oppose IPL activity by guiding DNMTs to target genes facilitating their suppression. Numerous studies over the last few years have revealed that lymphocytes also express unique profiles of lncRNAs that coordinate the development and activation state of both T and B cells. For example, the IFNγ locus is epigenetically regulated by NeST, a WDR5-interacting lncRNA [15]. IPLs facilitate the robust transcription of innate immune genes [36]. Therefore, it is highly likely that IPLs regulate robustly transcribed lymphocyte-specific genes that underlie adaptive immune responses.

Collectively, these studies have revealed numerous metabolic and epigenetic targets that could potentially be targeted to either induce or inhibit trained immunity. This would permit the ability to enhance the activity of the innate immune system in situations in which immune responsiveness of an individual is compromised, such as in patients who develop immune paralysis after sepsis, or people suffering from immunodeficiencies, AIDS, or certain types of cancer. On the other hand, the inhibition of the mechanisms involved in the induction of trained immunity would improve

the outcome in diseases characterized by excessive inflammation, such as atherosclerosis, diabetes, or inflammatory bowel disease, amongst others. The development of new materials such as nanoparticles, polymers, and supramolecular systems, that support the design of new therapeutic strategies to modulate the metabolic and epigenetic branches of trained immunity, is becoming a reality [41,42] and guarantees exciting developments in this field in the upcoming years.

Author contributions

J.D.A and S·F wrote the original draft. S.F. prepared the figures. L.A.B.J., M.M.M, and M.G.N. supervised the whole process and made revisions.

Conflict of interest statement

Nothing declared.

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