RESEARCH HIGHLIGHT

Inscribing the core memories of killers

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Recent studies on natural killer (NK) cells have challenged the dogma that immunological memory is a phenomenon restricted to the adaptive immune system. Specific interactions between the NK cell receptor Ly49H and the murine cytomegalovirus virus (MCMV)-encoded glycoprotein m157 result in selective expansion of the Ly49H + NK cell subpopulation and the establishment of a long-lived memory-like population.^{1–3} Further, memory NK cells show augmented IFN γ responses, and transfer of the memory population into naive mice confers enhanced protection against infection-induced mortality.¹ These observations along with cytotoxicity and cytokine secretion functions that overlap with CD8 T cells have led to the description of NK cells as a bridge between the innate and adaptive immune systems.⁴ Despite their common features, important distinctions exist between the specificity, kinetics, activation, and efficacy of NK and CD8 T cell responses. Furthermore, while the chromatin landscape of naive, effector, and memory CD8 T cells is increasingly well defined,⁵ less is known about changes in gene accessibility that occur as NK cells gain memory properties. A recent report by Lau et al. uncovers key epigenetic changes that are associated with the formation of memory NK cells and lays the foundation for the identification of a core epigenetic signature shared by memory NK and T cells.⁶

To understand the changes in gene accessibility and expression that occur as naive NK cells become activated, develop effector capabilities, and ultimately transition into memory, Lau et al. performed the assay for transposase-accessible chromatin using high-throughput sequencing (ATAC-seq) in parallel with RNA-seq on splenic Ly49H + NK cells procured at distinct time points during the response to MCMV. The analysis reveals both transient and long-lasting changes in gene accessibility. Not surprisingly, the bulk of the epigenetic remodeling in NK cells occurs during the first several days following infection and generally matches changes in transcriptional activity. These early modifications in chromatin architecture during this formative phase not only permit the production of effector molecules necessary for the NK cell-mediated clearance of MCMV, but also begin to imprint memory traits as the cells clonally expand and differentiate (Fig. 1).

Interestingly, during this initial period the chromatin is most dramatically changing at putative enhancer elements within introns and intergenic regions rather than at promoter sequences. This suggests that, unlike CD8 T cells which acquire progressive gain of access in promoters,⁵ NK cells rely more on changes in enhancer accessibility to drive gene expression as the cells evolve from naive to effector and memory states. In fact, naive NK cells show much higher levels of gene accessibility than naive T cells, and the ATAC-seq analysis clusters naive NK cells closer to

memory T cells rather than naive or effector T cells. Thus, naive NK cells may be more predisposed to respond, possibly reflecting their key innate surveillance roles. Similarly, helper-like innate lymphoid cells do not undergo large-scale chromatin remodeling once they are developmentally mature, even upon stimulation, whereas their adaptive counterpart, CD4 T cells, require activation to initiate remodeling at effector gene loci.⁷

The activation of NK cells is provoked in part by the presence of inflammatory cytokines including type I interferons and IL-12. These cytokine signals are conveyed by STAT1 and STAT4, respectively, and both of these molecules are required for the expansion of Ly49H + NK cells, as well as for the generation of NK cell memory. Lau et al. probe how these essential signaling molecules direct the epigenetic changes in the responding NK cells. By identifying STAT1 and STAT4 binding sites using chromatin immunoprecipitation sequencing (ChIP-seq) they nicely show distinct and antagonistic effects of these factors on the activation-induced remodeling of NK cell chromatin. Within the first 2 days following infection, the accessibility of STAT4 bound regions, primarily located within putative enhancer regions, changes dramatically. In contrast, STAT1 binding sites are more frequent in promoter regions and only mild changes in accessibility are observed at these sites. Furthermore, while gains in accessibility were noted for STAT4 binding sites, this was not the case for STAT1 sites, which generally became less accessible. Differences in chromatin accessibility between wild type and STAT1- or STAT4-deficient NK cells were also simultaneously compared using mixed bone marrow chimeras. These results support the notion that STAT4 operates to increase access at non-promoter regions, as STAT4-deficient NK cells displayed a relative net loss of accessibility at these putative enhancer sites when compared with wild-type NK cells. STAT1-deficient NK cells, however, displayed a decrease in accessibility in promoter regions, consistent with its binding preferences, but also showed increased chromatin accessibility in intronic and intergenic regions. Since there was apparent overlap between STAT1 binding sites and differentially accessible chromatin regions, it will be intriguing to determine how STAT1 controls the remodeling at these positions. Collectively, the findings support a model in which STAT1 and STAT4 convey early activating signals which differentially affect epigenetic changes that direct memory NK cell formation.

As expected, many changes in the chromatin landscape of NK cells manifest early following infection, during the first 7 days. Transient changes subside by day 14 and the alterations that remain at this point are largely maintained up until day 35, the last time point checked. By this time, the transcriptional profile has returned towards baseline and resembles, but is not identical to, that of naive NK cells (Fig. 1). Nevertheless, these memory NK cells

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Fig. 1 MCMV-reactive NK cells undergo changes in gene accessibility and expression as they transition from naive to effector and memory states. Lau et al., demonstrate that, upon activation, naive NK cells coordinately change gene accessibility and expression to promote effector phenotype and functions. The epigenetic landscape established by day 14 post-infection is then largely maintained, although gene expression patterns continue to adjust as homeostasis is restored. This results in a population of memory cells that is epigenetically poised to mount anamnestic recall responses

retain features of the “effector” chromatin landscape and appear poised for reactivation and ready to mount an accelerated recall response. This is in line with studies of “trained immunity” in monocytes and macrophages, which also undergo significant epigenetic modifications that allow for expeditious responses to secondary challenges.⁸ In the case of NK cells, the majority of the critical chromatin changes manifest as the cells initially respond. This implies that the epigenetic indoctrination that imprints the features of NK cell memory develops early and is then maintained by the cells that survive into the memory phase.

Comparative analysis of chromatin accessibility and transcription changes between naive and memory NK cells and CD8 T cells allowed Lau et al. to identify a common epigenetic signature of immunological memory. The majority of differentially accessible genes were detected only in CD8 T cells, with a few unique changes detectable in memory NK cells. This likely reflects the more extensive remodeling that occurs as T cells transition from naive to effector and memory states, but also suggests that certain changes specifically transpire in T cells that influence their memory properties, functional status, and longevity. Importantly, of the differentially accessible epigenetic peaks apparent in memory compared to naive cells 21% were common between both NK and CD8 T cells, whereas 9% of differentially expressed transcripts overlapped. Further assessment of the common epigenetic and transcriptional changes revealed a core signature of 94 genes that may be responsible for unified memory properties. This includes several transcription factors such as, *Bach2*, *Tcf7*, and *Zeb2*, that are known to influence CD8 T cell memory formation. Nevertheless, key distinctions remain in the properties of memory NK cells and CD8 T cells. Substantial

numbers of memory CD8 T cells are maintained at remarkably stable levels for years following their initial priming. Memory NK cells do not seem as stalwart and fall below detectable levels by 4–5 months, but do persist at low levels as evidenced by their ability expand in response to MCMV rechallenge.³ The comparative analysis of gene accessibility and expression presented by Lau et al. provides an excellent foundation for revealing the epigenetic basis responsible for both the overlapping and distinguishing features of memory NK and CD8 T cell populations.

This intriguing report⁶ begins to establish a core molecular signature of immunological memory that is common to adaptive CD8 T cells and NK cells. In the case of NK cells, the epigenetic changes which etch memory properties appear early in the course of the response to infection. Although regions of differential accessibility have now been documented it will be of interest to determine the precise mechanisms and modifications that are responsible for inducing these changes. Inflammatory signals play a central role in orchestrating NK cell activation and Lau et al. have shown that STAT1 and STAT4 have distinct roles in sculpting the chromatin landscape as the Ly49H⁺ NK cells respond to MCMV. This has profound consequences for the ultimate developmental fates and contributions of the NK cells to immediate infection control and longer-term protection to re-exposures. Thus, better understanding on how NK cell signaling networks integrate may uncover targets that can be manipulated in order to specifically direct the traits and longevity of the response. The definition of a common memory core gene signature provides an exciting opportunity to now investigate which of these constituents are essential for memory, what properties they confer, and how they act in concert to promote memory formation, survival, and host protection.

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ADDITIONAL INFORMATION

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