

Immune genes outside immune cells for multiple sclerosis

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In this issue of *Neuron*, Meijer and Agirre et al. (2022) demonstrate that immune genes exhibit a primed chromatin state in healthy oligodendroglia and are transcriptionally activated in MS through a series of epigenetic activations including histone modification deposition, transcription factor binding, and chromatin reconfiguration.

Immune genes are implicated in multiple and diverse disorders including obesity, schizophrenia, Alzheimer's disease, diabetes, cancer, multiple sclerosis (MS), and many autoimmune disorders. The recognition of these immune roles has come from both classical molecular experiments and from the recent coupling of large-scale epigenomic atlases with genome-wide association studies, showing enrichment of genetic variants associated with diverse, traditionally non-immune, disorders (Kundaje et al., 2015).

It is often assumed that immune genes function in classical immune cell types, including innate and adaptive immune cells. However, recent evidence indicates that immune transcriptional programs are also epigenetically primed and activated in non-immune cell types, including: structural endothelial, epithelial, and fibroblast cells in pathogen response (Krausgruber et al., 2020); intestinal stem cell renewal and differentiation by cytokine stimulation (Biton et al., 2018); and neurons, macroglia, and vascular brain cell types in Alzheimer's Disease (Garcia et al., 2022; Sun et al., 2022; Zalocusky et al., 2021). Previous studies showed that oligodendrocyte precursor cells (progenitors) could transit to an immune-like state in MS and then present antigens to T cells (Falcão et al., 2018; Fernández-Castañeda et al., 2020; Kirby et al., 2019). However, it is unclear: (1) whether the activation of the immune program in progenitors follows a similar epigenetic priming mechanism; and (2) whether those immune genes can be targeted by MS-associated SNPs.

In this issue of *Neuron*, Meijer, Agirre, and colleagues (Meijer et al., 2022) sys-

tematically characterize the gene-regulatory landscape of chromatin priming, histone modification, transcription factor binding, and transcription factor activation to address these two questions at single-cell resolution in both human samples and the experimental autoimmune encephalomyelitis (EAE) mouse model of MS. First, measuring single-cell chromatin accessibility in the EAE mouse model, the authors found that oligodendroglia, including oligodendrocyte progenitor cells and mature oligodendrocytes, showed distinct chromatin accessibility profiles compared to control mice. Genes nearest to higher-accessibility peaks in the MS mouse model oligodendroglia were enriched in immune pathways. Compared to microglia-specific immune signaling, the chromatin of genes involved in interferon responses was also accessible in the disease state, suggesting the potential importance of interferon signaling in MS oligodendroglia. The authors further showed increased chromatin accessibility at promoters and enhancers of a subset of immune genes in disease-specific oligodendroglia and correlated changes in accessibility between enhancers and nearby promoters. The authors classified genes into different categories based on differences in gene expression and chromatin accessibility between disease and control mice. They found that a subset of immune genes in oligodendroglia were in a primed chromatin state in control mice and later increased expression in the disease context. Even more chromatin accessibility was found in progenitor cells at inflammatory genes, indicating both broad and stage-specific

chromatin priming of immune genes in oligodendroglia.

The authors previously demonstrated that interferon induces expression of immune genes in oligodendrocyte progenitors (Falcão et al., 2018). In the current publication, they measured bulk-level chromatin accessibility and expression in progenitors with and without interferon treatment to understand the regulatory mechanism underlying increased expression of primed immune genes in disease oligodendroglia. The authors showed that interferon treatment induced expression of a large group of genes but few changes in chromatin accessibility, suggesting that interferon stimuli primarily regulated transcriptional rather than epigenetic states. The authors also found few similarities of transcriptional response between *in vivo* disease progenitors and *ex vivo* interferon-treated progenitors, likely stemming from the more complex *in vivo* environment of disease progenitors. This implies that interferon treatment might not be an optimal proxy for studying mechanisms of immune-gene response in disease progenitors. This issue could be addressed by using an *ex vivo* system that combinatorially uses systematically predicted potential stimuli, including interferon and other cytokines.

Using regulator activity and motif analysis in mouse accessibility data, the authors predicted a subset of regulators with well-known immunoregulatory functions and either enriched motifs or higher activity in disease oligodendroglia. The authors knocked down two of these immune regulators (BACH1 and STAT1) in interferon-treated progenitors to study their roles in immune gene transcription



in disease oligodendroglia. *Bach1* knockdown led to increased expression of immune genes, including major histocompatibility locus genes, indicating a repressive role of interferon-induced immune gene expression in progenitors. In contrast to *Bach1*, *Stat1* knockdown reduced the expression of some immune genes, suggesting that STAT1 contributed to the interferon-mediated upregulation of immune genes in oligodendrocyte progenitors. This was confirmed by the increased binding of STAT1 in interferon-treated progenitors. Collectively, these data indicate the importance of BACH1 and STAT1 in the transcriptional regulation of immune genes in interferon-treated oligodendrocyte progenitors and potentially in EAE oligodendrocytes. However, the directionality of causal relationships between disease state, interferon activation, transcription factor activity, and regulation of immune genes is not fully resolved, and additional directional perturbation experiments will be needed to resolve their potentially mutual relationships.

In addition to transcription factors, the authors profiled H3K27ac histone modifications indicative of active enhancers and promoters and found increased promoter and enhancer activity for immune genes. They also found increased binding of CTCF, a mediator of promoter-enhancer looping interactions, at promoters and enhancers of immune genes. Using experimental and computational methods to infer chromatin looping, Meijer et al. (2022) detected enhancer-promoter contact changes for differentially expressed genes upon interferon treatment in oligodendrocyte progenitors, including for the activated immune genes. These results expand their chromatin accessibility results, indicating additional gene-regulatory stages of activation of immune genes in oligodendrocyte progenitors.

The authors also profiled activation-associated H3K4me3 and repression-associated H3K27me3 marks in bivalent promoters and found that interferon treatment induced loss of repression and gain of activation marks for MHC-I and -II genes in non-MS progenitor oligodendrocytes. Inhibition of the Polycomb-associated enzyme that deposits H3K27me3 led to upregulation of a sub-

set of immune genes in progenitors, and interferon treatment led to expression of additional immune genes, including MHC and cytokine signaling genes. These results are a very nice demonstration of the expected roles of these well-established regulators of chromatin and gene expression changes in their primary culture system.

To explore whether their observations in their mouse model of MS and interferon-treated mouse oligodendrocyte progenitors also hold in human brain, the authors investigated chromatin accessibility of immune genes in two control human samples using single-nucleus multiomic joint accessibility and expression profiling. They found that MHC-I promoters were accessible in multiple cell types, including microglia, oligodendrocyte progenitors, mature oligodendrocytes, astrocytes, and neurons, even though these genes were not expressed in most cell types. The authors aligned reciprocal non-coding loci between human and mouse. They showed conserved accessibility between the two species and that their mouse-differential accessible sites in their MS model and interferon treatment mapped to human regions enriched for MS susceptibility SNPs. The strongest enrichments were for microglia-accessible sites, as expected, but oligodendroglia-accessible sites also showed a significant, albeit weaker, enrichment. Surprisingly, however, the authors found that MS-associated variants were most enriched in astrocyte- and oligodendrocyte-accessible sites, while microglia-accessible sites showed significantly less enrichment, contrary to their mouse results and contrary to human studies (Kundaje et al., 2015). This inconsistency in enrichments for their human data is surprising and could be due to their small sample size.

Despite differences between species and experimental settings, the enrichment of chromatin accessibility with MS variants was also observed in oligodendrocyte progenitors with interferon treatment. Using the *ex vivo* system of interferon-treated progenitors, the authors also assessed the chromatin landscape and transcription of MS-associated genes and variants. They found that some promoter-enhancer connections with MS-associated SNPs were established or

increased upon interferon treatment. For example, a connection from MS-associated SNP rs7191700 was formed to the *Socs1* promoter, along with increased chromatin accessibility and expression. Additionally, interactions between MS-associated SNP rs2248137 and the *Bcas1* (a key gene in early myelination) promoter were increased, with lower expression of *Bcas1* after interferon treatment. These results suggest that interferon treatment could affect the cellular states in the EAE mouse model of MS by altering the chromatin landscape and architecture, histone modification deposition, transcription factor binding, and gene expression.

Despite the elucidation of the epigenomic mechanisms of immune gene activation in oligodendroglia, several questions remain unanswered. What are the stimuli *in vivo* that trigger changes of immune epigenetic programs in non-immune cells? Which cell types are communicating with oligodendroglia to induce immune gene activation in oligodendroglia? Are the activated and/or disease-associated immune cells peripheral (e.g., T cells, B cells) or CNS-resident (e.g., microglia)? And lastly, do MS-associated SNPs contribute to the chromatin priming and accessibility increases in immune genes of non-immune cells?

Lastly, it is important to note that Meijer et al. (2022) only demonstrate the possibility that immune genes and pathways may *also* be acting in non-immune cells. The authors do not show that these non-immune-cell changes actually impact MS, and if such an impact does exist, the authors do not show that it is not mediated by immune cells (Figure 1). In fact, one could argue that evolutionary pressures have not excluded immune genes from also being active in non-immune cell types, where their activation might be inconsequential. However, the hypothesis raised in Meijer et al. (2022) is intriguing, in that at least some of the effects of genetic and environmental factors contributing to MS may also be mediated by non-immune cell types. Given the widespread activity of immune genes and control elements in many additional cell types, immune gene co-option for both immune and non-immune functions in non-immune cell types is a compelling possibility. It is therefore important to

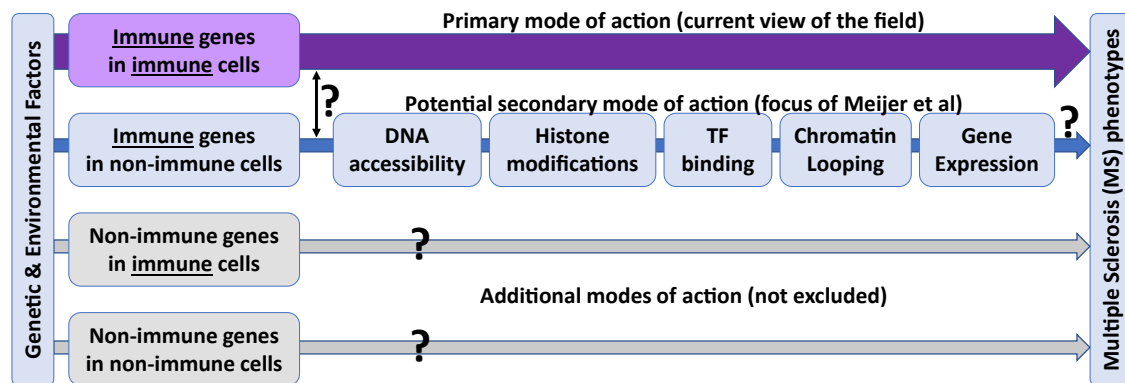


Figure 1. Immune and non-immune paths to MS

Diverse lines of evidence indicate that genetic and environmental factors contributing to MS primarily act through immune genes and pathways in immune cell types (top row). Meijer et al. demonstrate that immune genes in non-immune cell types also show changes in DNA accessibility priming, histone modifications, transcription factor binding, chromatin looping, and gene expression (second row). However, it is unclear whether these changes ultimately contribute to MS and whether they act independently of immune genes in immune cell types (question marks). Of course, neither previous studies, nor Meijer et al.'s study exclude the possibility that non-immune genes may also contribute to MS, both in immune and in non-immune cells (bottom rows).

test their causality in both MS and other immune-related and non-immune disorders.

In summary, Meijer and colleagues elucidate the epigenetic mechanisms of how non-immune cells (oligodendroglia) present immune gene functions in the context of a mouse MS model, using high-throughput genomic, epigenomic, and perturbation approaches. Meijer et al. (2022) provide important evidence for a potentially universal phenomenon: environmental stimulation priming immune epigenomic programs in non-immune cell types, potentially leading to a secondary immunological function of non-immune cells, even though causality and directness of these observations remain uncharacterized. More broadly, this work highlights that disease-associated SNPs likely also function outside of driver cell types, reminding researchers to consider a broader set of mechanisms for future studies.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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