Biological Psychiatry

Fearing the Mother's Virus: The Lasting Consequences of Prenatal Immune Activation on the Epigenome and Brain Function

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Adverse in utero environments can significantly impact brain structure and function, the consequences of which may extend from birth into adulthood and contribute to behavioral and mental disorders. DNA methylation, a key epigenetic mechanism, has emerged as a likely mediator of these long-term environmental effects on the brain (1). DNA methylation is essential for the establishment of correct gene expression programs during brain development and, hence, is very vulnerable to disruption. Multiple prenatal environmental exposures associated with increased risk for psychiatric disorders (e.g., stress, toxicants, environmental pollutants, drugs) have been shown to impact DNA methylation levels and gene expression in the brain in both the short term and long term. The epigenetic effects of early-life environments can persist into adulthood either through the maintenance of developmentally established, aberrant DNA methylation patterns (2) or, alternatively, as the consequence of improper programming of the brain's DNA methylation machinery that continues to be used by mature, postmitotic brain neurons (3).

Recent reports of congenital brain damage in newborns of Zika virus–infected mothers only remind us of the possible dramatic effects that maternal infection can have on brain development. However, gestational viral infections can lead to more subtle brain changes in offspring that may present as brain disorders decades following the infection. Multiple epidemiological studies have drawn links between maternal viral infections (e.g., influenza, herpes simplex) during pregnancy and an increased offspring's risk for psychosis-associated disorders, particularly schizophrenia, in adulthood (4). While schizophrenia is a complex syndrome encompassing multiple brain domains, increasing evidence shows that schizophrenia has a neurodevelopmental origin with globally dysregulated DNA methylation patterns in the brain that are thought to contribute to its development (5,6). While studying schizophrenia in human clinical populations and using postmortem human brain tissue is indispensable, there is a limit to which these approaches can provide mechanistic insights and tackle the question of causality. The big challenge is how to mechanistically connect early-life environmental, infectious, or other insults to schizophrenia-related pathology that only starts to emerge at some postpubescence time point to better understand the mechanisms underlying initiation and maintenance of this disorder. To this end, animal studies have just started to explore a possible role of epigenetic mechanisms in mediating long-term neurobehavioral effects of prenatal immune activation (7,8).

A study by Richetto *et al*. (9) now provides important evidence that DNA methylation–dependent mechanisms may underlie the long-term consequences of immune activation on brain function. The authors used an established mouse model of prenatal viral-like immune activation—a single injection of polyriboinosinic-polyribocytidylic acid, an analog of double-stranded RNA, to pregnant dams. Considering possible critical windows of vulnerability to infectious insults, two developmental windows were examined: midgestation (gestational day [GD] 9), corresponding to gestational weeks 4–5 in humans; and late gestation (GD17), corresponding to human gestational weeks 28–29 (Figure 1). Richetto *et al*. first explored the effect of prenatal immune activation on adult behavioral phenotypes including social interaction, sensorimotor gating, and spatial recognition memory. Each of these cognitive-behavioral domains is affected in schizophrenia and related neurodevelopmental disorders. Not surprisingly, the behavioral effects of immune activation during midgestation and late gestation show only partial overlap, as both treatments induce social interaction deficits (Figure 1). The midgestation but not late-gestation polyriboinosinic-polyribocytidylic acid injection led to impaired sensorimotor gating (implying an important window for sensorimotor development), whereas only the late-gestation treatment affected spatial memory (suggesting a window for the development of working memory-related circuits). To explore an involvement of epigenetic mechanisms, following behavioral testing, prefrontal cortices (PFCs) of control and immune-challenged animals were examined for DNA methylation using a targeted, nucleotide-resolution genome-wide assay covering around 3.7 million CpG sites (~16% of the methylome).

Maternal immune activation induced long-term, genome-wide DNA methylation changes in the offspring's PFC, which were largely dependent on the timing of exposure, consistent with the time-specific behavioral effects. Both hyper- and hypomethylation changes were observed at both time points and at distinct genomic regions, including promoters, introns, exons, and intergenic regions. A total of 2365 CpGs (1408 genes) and 3361 CpGs (1756 genes) were differentially methylated in the midgestation and late-gestation time points, respectively, with only 335 overlapping sites (encompassing 167 genes) (Figure 1). Importantly, the DNA methylation changes induced at both time points were enriched at genes involved in neuronal differentiation. Differentially methylated regions following the GD9 immune-activation were primarily associated with genes involved in Wnt signaling, which is necessary for both brain development and adult synaptic plasticity. The GD17 immune activation was primarily associated with DNA methylation changes in genes involved in the differentiation of the gamma-aminobutyric acid (GABA)ergic

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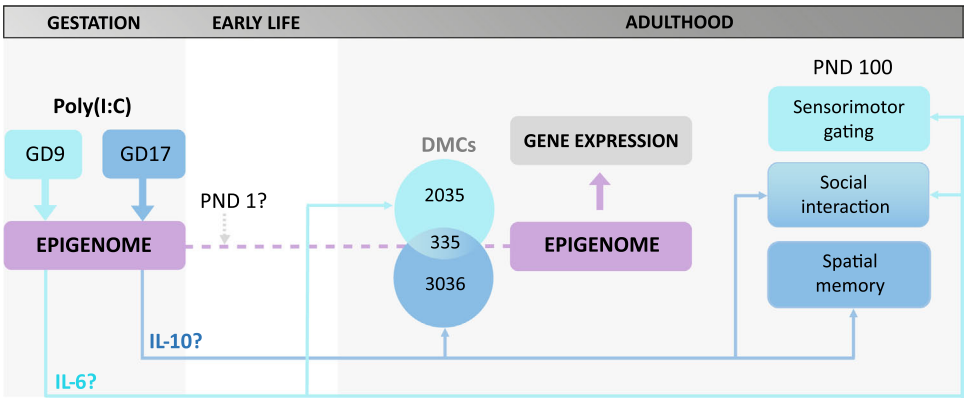

Figure 1. Prenatal immune activation effects on the brain epigenome and function. Prenatal immune activation using polyriboinosinic-polyribocytidylic acid [poly(I:C)] in two separate developmental windows, midgestation (gestational day [GD] 9, light blue) and late gestation (GD17, dark blue), induces widespread DNA methylation and gene expression changes in adulthood (postnatal day [PND] 100), which are gestational window specific and showing only partial overlap, consistent with time-specific behavioral changes. Genes affected in adulthood were not impacted at PND1. Color-coded lines show the effects of each gestational exposure on the epigenome and behavior. DMCs, differentially methylated CpGs; IL, interleukin.

neuronal system. Among genes affected by both treatments are those previously implicated in neurodevelopmental disorders: neuregulin 1 (*Nrg1*), neurexin 2 (*Nrxn2*), and neuronal differentiation 6 (*Neurod6*). For selected genes, experimental confirmation that the DNA methylation differences correlated with changes in gene expression further suggested that the altered methylation levels were functionally relevant.

An important question is whether the induced epigenetic changes, even if associated with transcriptional changes, explain the observed phenotypes and the differences between the two exposures. The current study was not designed to directly address this question and additional studies will be needed to further assess causality. However, there is some supporting evidence relevant to this point. First, previous studies have shown that immune activation at both time windows affects GABAergic neuronal function. The late prenatal exposure has more overt effects on GABAergic cell morphology and gene expression, which may in part be explained by the more profound DNA methylation changes observed in GABA-relevant genes following G17 exposure. Importantly, DNA methylation changes in GABAergic genes are implicated in GABAergic deficits and cognitive impairments associated with schizophrenia (5), which is consistent with epigenetic changes and cognitive deficits associated with GD17 immune activation. There is also limited evidence supporting a role for the Wnt-signaling pathway in sensory development and schizophrenia (10), implying that DNA methylation changes in Wnt signaling–related genes following GD9 immune activation may in part be responsible for specific sensorimotor effects of the midgestation infection and could provide a possible link between this gestational exposure and deficits observed in schizophrenia.

The second important question relates to the possible mechanisms that underlie the observed DNA methylation changes. DNA methylation changes are likely downstream effects of specific pathway(s) activated by the viral mimetic, and it is plausible that factors involved in the immune response play a crucial role. Based on previous findings, the authors speculate that cytokines are likely mediators and that, interleukin 6, in particular, may be involved in the midgestation immune activation while interleukin 10 may play a major role in the late-gestation stage activation (Figure 1). These cytokines have already been associated with epigenetic effects although their roles in regulating the brain methylome still need to be explored. It is important to note that genes differentially methylated in the adult PFC were not differentially methylated in the PFC at postnatal day 1 of the prenatally immune-challenged animals. This is in contrast with earlier studies showing persistent DNA methylation changes induced by early-life environmental factors (2). However, considering the number of assessed loci, this is not very surprising. The neuronal DNA methylome is dynamic and plays an important role in activity-regulated transcription in later life stages including adulthood. While certain genes may harbor persistent DNA methylation changes over long periods of time, it is likely that the number of methylated sites will be dynamically regulated. Therefore, an early-life insult may affect the developmental programming of the epigenetic machinery that regulates DNA methylation throughout life, while the DNA methylation status of genes may be age and state dependent. It is, therefore, important to understand how cytokines could permanently affect the brain's epigenetic machinery (e.g., DNA methyltransferases and Tet proteins) to induce lasting effects on the dynamic brain methylome.

In summary, Richetto *et al.* provide important evidence that prenatal immune activation induces genome-wide changes in the brain methylome, which persist into adulthood and affect gene expression. The study provides a possible link between prenatal immune activation and widespread DNA methylation changes found in neurodevelopmental disorders including schizophrenia. However, many questions remain and warrant future studies that may include 1) additional postnatal time points to better understand the timing of the immune challenge and DNA methylation changes; 2) assessment of the causal relationship between DNA methylation changes and behavioral phenotypes; 3) assessment of both sexes, as the methylomes are sex specific; 4) increased DNA methylation coverage because unassessed genomic regions are likely relevant; 5) hydroxymethylation and other epigenetic modifications, which are likely involved; 6) cell type–specific epigenomic analyses to increase accuracy and resolution of the epigenomic profiling; 7) analysis of additional brain regions, as the PFC is likely one of many brain regions affected; and 8) DNA methylation analyses of peripheral tissues that can

be explored as possible biomarkers in humans. This study opens an important avenue in our search to better understand neurodevelopmental disorders and how to intervene early to prevent and alleviate suffering associated with these debilitating disorders.

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