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Chorioamnionitis exposure remodels the unique histone modification landscape of neonatal monocytes and alters the expression of immune pathway genes

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Abstract

Chorioamnionitis is an intrauterine infection involving inflammation of the chorion, amnion and placenta. It leads to a fetal systemic inflammatory response that can alter the transcription of neonatal immune genes. We have previously shown that neonatal monocytes gain the activating histone tail modification H3K4me3 at promoters of immunologically important genes as development progresses from preterm neonate to adult. In this study, we applied ChIP-seq and RNA-seq to evaluate the impact of chorioamnionitis on the neonatal monocyte H3K4me3 histone modification landscape over the course of fetal and neonatal immune system development. Chorioamnionitis exposure in neonatal monocytes resulted in a net increase in total monocyte H3K4me3, primarily in introns and intergenic regions. Immune gene expression was decreased in chorioamnionitis-exposed monocytes, with the majority of enriched transcripts falling into pathways that are not linked to the immune system. Over half of all neonatal monocyte H3K4me3 peaks, independent of their location, were associated with active gene transcription. Overall, chorioamnionitis exposure resulted in global remodeling of the neonatal monocyte H3K4me3 landscape and changes in the expression of known immune genes. These changes resulted in a less robust inflammatory response upon exposure to a secondary challenge, which may explain why chorioamnionitis-exposed neonates have an increased risk of sepsis.

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Author Contributions

JB conceptualized the study, planned and performed the experiments, analyzed the data and drafted the manuscript. KG contributed to the data analysis and interpretation and revised the manuscript for critical intellectual content. AD contributed to the data analysis and interpretation and revised the manuscript for critical intellectual content. SK contributed to experimental design, provided needed reagents and resources and revised the manuscript for critical intellectual content. NL helped to conceptualize and design the study, provided needed reagents and resources, assisted with data interpretation and revised the manuscript for critical intellectual content. MS assisted with experiment conceptualization and design, data analysis and interpretation and revised the manuscript for critical intellectual content.

Conflicts of interest: The authors have no conflicts of interest to report.

Databases:

ChIP-seq data for U30/O30/Term: GEO GSE81957

ChIP-seq data for U30C/O30C/TermC: GEO GSE111873

RNA-seq data for U/L/CU/CL: GEO GSE111927

Graphical Abstract

Exposure to chorioamnionitis, a common intrauterine infection, alters the neonatal monocyte histone modification landscape. Specifically, the activating histone modification mark H3K4me3 is removed from promoters and instead enriched in introns and intergenic regions. This results in reduced expression of immune pathway genes, leading to a decreased inflammatory response when a secondary immune stimulus is encountered.

Keywords

chorioamnionitis; histone modification; monocyte; transcription; epigenetics

Introduction

Chorioamnionitis is a condition involving infection and inflammation of the chorion, amnion and placenta and is the most common cause of preterm birth, although it complicates up to 4% of term births as well [1, 2]. Chorioamnionitis exposure can lead to a fetal systemic inflammatory response that alters the developing immune system and places exposed infants at a higher risk of developing early onset sepsis [3–5]. It is unclear if this sepsis risk is due to a common infectious agent leading to both conditions or as a result of remodeling of the neonatal immune system that leaves exposed infants vulnerable to infections. Recent studies demonstrate that exposure to histologic chorioamnionitis alters the neonatal immune transcriptome, with activation of innate immune pathways [6, 7]. Chorioamnionitis exposure can also have long-term consequences for exposed infants, including neurodevelopment impairment, cerebral palsy and the development of immune-mediated diseases later in life, including wheezing and asthma [8–11]. This highlights the importance of perinatal exposures on the developing immune system, which may result in life-long alterations in immune responses.

Monocytes are first responders of the innate immune system, sensing pathogens and initiating an inflammatory cascade that results in the clearance of microbes[12]. Neonatal monocytes demonstrate major deficiencies in chemotaxis, microbial killing and pro-inflammatory cytokine expression, all of which contribute to a heightened risk of infection early in the neonatal period[13–15]. Epigenetics refers to modifications of DNA structure and chromatin accessibility that alter gene expression without affecting the underlying genetic code. Many changes to DNA structure occur as the result of histone tail modifications, which bind to DNA and package it into chromatin. The addition of three methyl groups to lysine (K) 4 on histone (H) 3 (H3K4me3) near gene promoters allows for active gene transcription[16]. We have previously shown that neonatal monocytes gain H3K4me3 at promoter sites of immunologically important genes, including *IL1B*, *TNF*, and *CCR2*, as development progresses from preterm neonate to adult[17]. This developmental change in the monocyte histone modification landscape was found to contribute to neonatal-specific immune responses that leave them vulnerable to infections. It is currently unknown how exposures in the perinatal period, including chorioamnionitis, alter the normal developmental progression of the histone modification landscape in the neonatal immune system, and what impact this has on monocyte transcription and function.

In this study we utilized chromatin immunoprecipitation followed by massively parallel DNA sequencing (ChIP-seq) to determine the impact of chorioamnionitis exposure on the neonatal monocyte H3K4me3 histone modification landscape as development progressed from extremely preterm to term neonate. We then performed whole transcriptome shotgun sequencing (RNA-seq) on unstimulated and lipopolysaccharide (LPS) stimulated monocytes from term healthy and chorioamnionitis-exposed neonates to evaluate the impact of chorioamnionitis exposure on monocyte transcription at baseline and upon a secondary inflammatory stimulus. These experiments revealed that chorioamnionitis exposure remodeled the neonatal monocyte H3K4me3 landscape and altered gene transcription, with decreased expression of known immune genes. Additionally, H3K4me3 presence near a known gene, not just at promoter sites, predicted mRNA expression over 50% of the time.

Results

Chorioamnionitis exposure altered the abundance and location of neonatal monocyte H3K4me3

To determine the influence of chorioamnionitis on the monocyte H3K4me3 landscape we performed ChIP-seq on preterm and term monocytes with and without exposure to histopathologic chorioamnionitis. We evaluated the global distribution of H3K4me3 in umbilical cord blood purified CD14⁺ monocytes from six different experimental groups: under 30-week extremely preterm healthy infants (U30), under 30-week extremely preterm chorioamnionitis-exposed infants (U30C), over 30-week preterm healthy infants (O30), over 30-week preterm chorioamnionitis-exposed infants (O30C), term healthy infants (Term) and term chorioamnionitis-exposed infants (TermC). Table 1 compares patient characteristics between the healthy and chorioamnionitis-exposed infants in each gestational age group. Overall, the groups for each age and exposure group were well matched for gestational age, birth weight, sex, maternal smoking, gestational hypertension and gestational diabetes. The

only difference noted was that infants in the O30C and Term groups were more likely to be born by vaginal delivery than those in the comparison groups. Fig. 1 summarizes differences in H3K4me3 abundance and location between these different experimental groups. A principal component analysis of monocyte H3K4me3 normalized read counts reveals that preterm healthy and chorioamnionitis-exposed monocytes cluster together along principal component 2, with some differences between preterm healthy and chorioamnionitis-exposed monocytes on principal component 1 (Fig. 1A). The term healthy sample replicates cluster together and the term chorioamnionitis-exposed sample replicates cluster together but are distinct from each other in both principal components 1 and 2 (Fig. 1A). We have previously reported an increase in total monocyte H3K4me3 peaks as development progresses from preterm to term neonate[17]. In addition to this developmentally related increase in monocyte H3K4me3, chorioamnionitis exposure in preterm and term monocytes significantly increases the total number of H3K4me3 monocyte peaks (Fig. 1B). To obtain a broad overview of H3K4me3 distribution, the human genome was divided into four distinct categories according to the UCSC Genome Browser: promoters, introns, exons and intergenic regions[18]. Chorioamnionitis exposure had little to no impact on the genomic location of H3K4me3 peaks in extremely preterm monocytes (Fig. 1C-E). Chorioamnionitis exposure resulted in decreased promoter/exon peaks in term monocytes (Fig. 1C). In contrast, exposure to chorioamnionitis resulted in increased intergenic H3K4me3 peaks in preterm and term monocytes with increased intron peaks in term monocytes (Fig. 1D-E). All gestational age and exposure groups had H3K4me3 enriched in gene ontology pathways necessary for basic cell survival and function, including cellular metabolism, cell signaling and maintenance of cell structure (Fig. 2A). Chorioamnionitis exposure had minimal impact on the gene ontology pathways enriched for H3K4me3 in extremely preterm monocytes. In contrast, chorioamnionitis exposure in preterm and term monocytes was associated with increased H3K4me3 enrichment in gene ontology pathways associated with development, with heavy enrichment in pathways important in nervous system development (Fig. 2A). The top gene ontology pathways enriched for H3K4me3 in healthy extremely preterm, preterm and term neonatal monocytes was previously published[17], and the top 10 H3K4me3 enriched gene ontology pathways in chorioamnionitis-exposed neonates are shown in Fig. 2B-D. Immunologically relevant gene ontology pathways enriched for H3K4me3 in chorioamnionitis-exposed monocytes are detailed in Table 2.

Chorioamnionitis exposure resulted in removal and deposition of H3K4me3 in monocytes at all gestational ages

We then derived consensus H3K4me3 peaksets for each gestational age and exposure group, and peaks needed to be present in two out of three replicates to be included. The H3K4me3 consensus peaks present in each gestational age and exposure group are outlined in Table 3. Next, we compared H3K4me3 peak locations (occupancy) between gestation and exposure groups. At all gestations there were unique H3K4me3 peaks present in both the healthy and chorioamnionitis-exposed monocytes, with varying amounts of shared H3K4me3 peak locations depending on the gestation (Fig. 3A-C). When the chorioamnionitis-exposed monocytes were compared between gestations, there were some common H3K4me3 peaks shared by all groups, with the largest overlap between the preterm (O30) and term groups (Fig. 3D). In the extremely preterm neonates there were no differences in average H3K4me3

peak width between exposure groups. In contrast, in the preterm and term populations the chorioamnionitis-exposed monocyte H3K4me3 peaks were narrower than those in the healthy monocytes (Table 4). Representative unique and common H3K4me3 peaks for the term population are shown in Fig. 4. Representative common H3K4me3 peaks for the chorioamnionitis-exposed monocytes are shown in Fig. 5. A binding affinity analysis was then performed to determine differentially bound H3K4me3 peaks between gestational age and exposure groups. A binding affinity heatmap demonstrated that term healthy monocytes and term chorioamnionitis-exposed monocytes had distinctly different H3K4me3 binding affinity patterns, while preterm healthy and chorioamnionitis-exposed monocyte H3K4me3 binding affinity was more varied (Fig. 6). An overview of differentially bound H3K4me3 sites between different gestational age and exposure groups is presented in Table 5 with a more comprehensive list in Supplemental Data. Representative differentially bound H3K4me3 peaks between term chorioamnionitis-exposed and unexposed monocytes are shown in Fig. 7. Gene ontology pathways enriched for differentially bound H3K4me3 between gestational age and exposure groups are detailed in Table 6. The most striking differences in gene ontology pathways enriched for differentially bound H3K4me3 were between term chorioamnionitis-exposed and unexposed monocytes. Term unexposed monocytes had increased H3K4me3 in a broad array of gene pathways, including protein catabolism, transcriptional regulation, apoptosis, response to virus, intracellular signaling and autophagy (Table 6). In contrast, although chorioamnionitis exposure resulted in an overall increase in monocyte H3K4me3 peaks, term chorioamnionitis-exposed monocytes had very few gene pathways enriched for H3K4me3, suggesting that chorioamnionitis stimulated H3K4me3 deposition in a more random and poorly regulated manner (Table 6).

Chorioamnionitis exposure changed neonatal monocyte transcription both in unstimulated and LPS stimulated conditions

In order to determine the impact of this chorioamnionitis induced global monocyte H3K4me3 landscape remodeling on gene transcription, we performed RNA-seq on a separate cohort of infants. Given the low H3K4me3 peak abundance in preterm monocytes, we chose to focus solely on term monocytes. Table 1 compares the patient characteristics between RNA-seq groups. The healthy and chorioamnionitis-exposed groups were well matched for gestational age, birth weight, sex, mode of delivery, maternal smoking, gestational hypertension and gestational diabetes. RNA-seq was performed on umbilical cord blood purified CD14+ monocytes from four different experimental groups: term healthy unstimulated monocytes (U), term healthy LPS stimulated monocytes (L), term chorioamnionitis-exposed unstimulated monocytes (CU) and term chorioamnionitis-exposed LPS stimulated monocytes (CL). RNA-seq of unstimulated and LPS stimulated monocytes was performed to determine if chorioamnionitis exposure impacted gene transcription in a resting state and if it altered gene transcription upon a secondary pathogenic stimulus. A principal component analysis of normalized transcript read counts revealed that the unstimulated chorioamnionitis-exposed and unexposed monocytes clustered together, the healthy LPS stimulated monocytes clustered together, but the chorioamnionitis-exposed LPS stimulated monocytes displayed significantly more variance (Fig. 8A). A pearson's correlation heatmap of normalized read counts of differentially expressed transcripts revealed that the differentially expressed transcripts were very consistent between replicates,

with separation between exposure groups (Fig. 8B-C). An overview of the differentially expressed transcripts between exposure and treatment groups is presented in Table 7 with a more detailed list in Supplemental Data. As expected, LPS stimulation of non-chorioamnionitis exposed monocytes resulted in increased mRNA expression in gene ontology pathways related to cytokine activity and the inflammatory response (Table 8). Of note, there was increased expression of the immune related genes *IL6*, *IFIT2*, *IFI44* and *CSF3* after LPS stimulation in the unexposed monocytes, which is consistent with previously published data[19]. In contrast, LPS stimulation of chorioamnionitis-exposed monocytes did not result in enriched transcript expression in any gene ontology pathways, although there was increased expression of the immune related genes *IL6* and *CSF3* (Table 8). This suggests that chorioamnionitis exposure results in a dysregulated monocyte inflammatory response when a secondary pathogen was encountered. Gene ontology pathways enriched for differentially expressed transcripts between chorioamnionitis-exposed and unexposed monocytes primarily involved oxygen and bicarbonate transport, with a large induction in hemoglobin associated genes in the chorioamnionitis-exposed monocytes, both in unstimulated and LPS stimulated conditions (Table 8). Network analysis of differentially expressed transcripts in either LPS stimulated healthy or chorioamnionitis-exposed monocytes showed significant overlap in KEGG pathways with gene enrichment in both groups, including response to infection and TGF-beta signaling (Figure 9A-B). Given the lack of gene enrichment in distinct immunologic pathways between chorioamnionitis-exposed and healthy monocytes, we sought to determine if there was a difference in monocyte function upon a secondary inflammatory exposure. To assess this, we measured inflammatory cytokine expression 24 hours after LPS stimulation. Both healthy and chorioamnionitis-exposed term monocytes had significantly increased expression of the pro-inflammatory cytokines IL-1 β , IL-6, IL-8 and TNF- α compared to the unstimulated controls (Fig 10A-D). There was very little IL-10 detected in either the unstimulated or LPS stimulated samples (Fig. 10E). There was decreased expression of the pro-inflammatory cytokines IL-1 β , IL-6 and IL-8 in chorioamnionitis-exposed term monocytes when compared to term healthy monocytes (Fig. 10A-C). This indicates that while there are no clear differences in transcript expression in distinct inflammatory pathways when monocytes with previous chorioamnionitis exposure encounter a secondary pathogen, there is a difference in monocyte function, particularly pro-inflammatory cytokine expression.

H3K4me3 peak presence in neonatal monocytes predicted RNA expression

We then took consensus H3K4me3 peaks that annotated to known genes and compared them to normalized transcript read counts to evaluate if the presence and location of H3K4me3 peaks around a gene was associated with transcript expression. RNA expression was considered present if the normalized read count was greater than 10 using the edgeR package[20]. In healthy term monocytes, the presence of an H3K4me3 peak within or around a known gene was associated with RNA expression 60.4% of the time in unstimulated cells and 58.7% of the time in LPS stimulated cells. In chorioamnionitis-exposed term monocytes the presence of an H3K4me3 peak within or around a known gene was associated with significantly less RNA expression, only 53.2% of the time in unstimulated cells and 50% of the time in LPS stimulated cells (Fig. 11A). Next, we evaluated if the ability of H3K4me3 peaks to predict gene expression was dependent on its

location, as H3K4me3 is known to be primarily located at promoter regions of actively transcribed genes[21]. Promoter H3K4me3 peaks in the healthy monocytes were associated with RNA expression less than 50% of the time, while promoter H3K4me3 peaks in the chorioamnionitis-exposed monocytes were associated with RNA expression over 50% of the time (Fig. 11B). Of note, there were few promoter H3K4me3 consensus peaks in the chorioamnionitis-exposed monocytes (92), with a significantly higher number in the healthy monocytes (292), making it difficult to draw any conclusions from these findings. In contrast, there were significantly more intron H3K4me3 peaks in the chorioamnionitis-exposed monocytes that were not associated with gene expression. Furthermore, significantly more intron H3K4me3 peaks in the healthy monocytes were associated with high level RNA expression (Fig. 11C). Interestingly, intergenic H3K4me3 peaks, which could be as distant as 800 kb from the gene of interest, predicted RNA expression approximately 50% of the time in all exposure and treatment groups (Fig. 11D). Overall, the presence of H3K4me3 near a gene predicted RNA expression approximately 50% of the time, independent of the actual location of the H3K4me3. Next, we compared differentially bound H3K4me3 peaks with differentially expressed mRNA transcripts between exposure and treatment groups (Table 9 and Table 10). Overall, approximately 10% of differentially expressed transcripts were associated with differentially bound H3K4me3 peaks. Conversely, less than 2% of differentially bound H3K4me3 peaks were associated with differentially expressed mRNA transcripts.

Discussion

Although chorioamnionitis is a frequent complication encountered around the time of delivery, there are few studies in humans that have investigated its effect on neonatal immune cell development and function. In this study we demonstrated that exposure to chorioamnionitis resulted in global remodeling of the monocyte H3K4me3 landscape, with both removal and deposition of H3K4me3 in extremely preterm, preterm and term neonates. In preterm and term neonates this remodeling resulted in an overall gain in monocyte H3K4me3, which was primarily occurring in introns and intergenic regions. We also demonstrated that chorioamnionitis exposure altered global term neonatal monocyte transcription, both at baseline and after a secondary pathogenic stimulus. Furthermore, monocyte H3K4me3 peaks located near known genes was associated with mRNA expression over 50% of the time, with better correlation noted in non-chorioamnionitis-exposed monocytes. However, differentially bound H3K4me3 peaks between healthy and chorioamnionitis-exposed monocytes were a poor predictor of differentially expressed mRNA.

We have previously demonstrated that neonatal monocytes gain H3K4me3 as development progresses from extremely preterm neonate to adult. We have also shown that the majority of H3K4me3 peaks in neonatal monocytes are located in intergenic regions while they are located in promoter and exon regions in adult monocytes[17]. Here we demonstrate that chorioamnionitis exposure resulted in a global remodeling of the neonatal monocyte H3K4me3 landscape, with both removal and deposition of H3K4me3 that resulted in an overall gain of H3K4me3 in preterm and term monocytes. Interestingly, this H3K4me3 gain was primarily in introns and intergenic regions, so although the total number of H3K4me3

peaks in term chorioamnionitis-exposed monocytes approximates that of adult monocytes, the location of the peaks and global H3K4me3 landscape were strikingly different. This demonstrates that the gain of H3K4me3 that occurs over the course of development from neonate to adult can be disrupted by a pathogenic inflammatory exposure in the neonatal period. We suspect that developmentally related H3K4me3 removal and deposition occur in a very nuanced manner, requiring a series of appropriately timed specific stimuli for this process to occur effectively. It is clear that exposure to chorioamnionitis disrupts this process, but the long-term implications of this are unknown.

The H3K4me3 peaks in preterm and term healthy monocytes are broader than the peaks in the chorioamnionitis-exposed monocytes of the same gestation. Broad H3K4me3 peaks, typically defined as greater than 1.5 kb, are associated with enhanced gene expression[22, 23]. In this study there is a significantly larger correlation between H3K4me3 peak presence and mRNA expression in term healthy monocytes (58–60% of H3K4me3 peaks are associated with mRNA transcript expression) when compared to term chorioamnionitis-exposed monocytes (50–53% of H3K4me3 peaks are associated with mRNA transcript expression). It is possible that the difference in H3K4me3 peak width between exposure groups is contributing to this difference in mRNA expression. H3K4me3 is an activating histone tail modification that is primarily believed to be located at promoter and 5'-coding regions of actively transcribed genes[21, 24]. It is interesting that upon chorioamnionitis exposure H3K4me3 is removed from promoter regions and deposited in introns and intergenic regions, where it does not have a well-described function. It is also surprising that H3K4me3 peaks predicted gene expression greater than 50% of the time regardless of its location. This demonstrates that H3K4me3 is not only associated with active gene transcription when it is located at promoter regions, but can also direct active gene transcription of nearby genes when located in introns and intergenic regions as well. It has been postulated that some 3' H3K4me3 peaks have a role in antisense transcription[25] and recent studies have demonstrated that non-promoter H3K4me3 can act as transcriptional enhancers in T-cells and cancer cell lines[26, 27]. Therefore, it is plausible that non-promoter monocyte H3K4me3 peaks can influence the expression of genes located far distances away, in part due to the three-dimensional structure and extensive folding of cellular DNA. It is unclear why differential H3K4me3 binding between exposure groups did not predict differential mRNA expression. It may be that the presence or absence of H3K4me3 is more important in influencing gene expression than smaller differences in abundance or affinity that differential binding algorithms highlight. Additionally, the ChIP-seq and RNA-seq experiments were performed on different patients, which may have contributed to this poor correlation. It is also likely that there are factors in addition to H3K4me3 influencing mRNA expression that were not accounted for in this study.

To date, studies evaluating the impact of chorioamnionitis exposure on human neonatal immune function have focused primarily on preterm neonates. It is true that chorioamnionitis is more prevalent in preterm births, but it still complicates up to 4% of term deliveries[2, 28]. Studies focusing on neonatal adaptive immunity have shown that chorioamnionitis exposure amplifies Th17 and Th1 type responses in preterm infants[29, 30]. A study focusing on both innate and adaptive immunity employed a broad transcriptomic approach, using a microarray to evaluate mRNA expression in whole blood of

preterm neonates with and without chorioamnionitis exposure[6]. While this approach revealed that chorioamnionitis exposure altered gene expression in both innate and adaptive immune pathways, the approach made it impossible to determine which cells were responsible for these differences. A more recent study performed RNA-seq on purified cord blood monocytes from very preterm neonates (<32 weeks) with and without chorioamnionitis exposure both before and after stimulation with the most common etiology of neonatal late onset sepsis, *Staphylococcus epidermidis*[7]. This study found that chorioamnionitis exposure in preterm infants is associated with a hypo-responsive transcriptional profile, which may alter a preterm infants' risk of sepsis. While chorioamnionitis in term pregnancies is a less common and more heterogeneous condition than chorioamnionitis in preterm pregnancies, it can still result in a significant perinatal inflammatory exposure that has the potential to impact neonatal immune function. We have provided evidence here that chorioamnionitis exposure in term pregnancies resulted in altered monocyte gene transcription, with less induction of genes involved in the inflammatory cascade, consistent with a hypo-responsive transcriptional profile. This translated to decreased pro-inflammatory cytokine expression when monocytes with previous chorioamnionitis exposure encountered a secondary inflammatory stimulus. Chorioamnionitis exposure was also noted to upregulate genes vital to oxygen and gas exchange, including numerous hemoglobin-associated transcripts. Suboptimal tissue gas exchange in hemoglobinopathies is thought to result in compensatory hemoglobin synthesis in different cell lineages, including monocytes[31]. Inflammation associated immune cell activation causes increased oxygen consumption and cellular respiration, so it is possible that the chorioamnionitis-exposed monocytes are upregulating hemoglobin-associated transcripts in an attempt to compensate for this[32].

Both term and preterm fetuses exposed to chorioamnionitis can mount a systemic inflammatory response, resulting in elevated fetal cortisol levels and fetal immune cell activation with increased expression of the cytokines and chemokines IL-1 β , IL-6, TNF- α , G-CSF and CXCL1[33–37]. There is also an association between chorioamnionitis exposure and the development of early onset neonatal sepsis that persists even after adjusting for confounding factors such as birth weight and gestational age[38–41]. This association has been attributed to either a common infectious agent leading to both conditions or as a result of immune system reprogramming that results in increased susceptibility to infection in the early neonatal period. A growing body of evidence points to immune system reprogramming, including the fact that maternal antibiotic administration does not impact the increased early onset sepsis risk associated with chorioamnionitis exposure[42, 43]. Our study also supports the immune system reprogramming hypothesis, and demonstrates that exposure to chorioamnionitis results in alteration in the neonatal monocyte transcriptome, both at baseline and with a more pronounced difference upon a secondary pathogenic stimulus. LPS was chosen as the secondary pathogenic stimulus as gram-negative organisms, primarily *Escherichia coli*, are some of the leading causes of early onset neonatal sepsis and are one of the earliest pathogenic exposures a neonate is likely to encounter[44]. It is currently unclear how long this chorioamnionitis induced immune system reprogramming lasts, as studies are conflicting about whether chorioamnionitis exposure is protective against or increases susceptibility to neonatal late onset sepsis, which occurs

beyond the first 72 hours of life[38, 45, 46]. Little is known about the impact of chorioamnionitis exposure on the susceptibility to other bacterial or viral infections in the neonatal, infant and childhood periods, although further studies specifically addressing this question would be informative.

We realize that this study has several limitations. All of the patients included in this study were recruited from a single center, which could have introduced bias into the results. We attempted to control for interpatient variability and genetic diversity by pooling patient samples and using separate patient groups for the ChIP-seq and RNA-seq experiments, but this approach may have masked the identification of important differences between individual patients. The purity of the CD14⁺ isolation was 92%, which could have resulted in non-monocyte population transcripts being included in the RNA-seq analysis. We do not believe this significantly impacted the main conclusions of the study as the non-monocyte cell populations included in the mononuclear cell layer after Ficoll separation are primarily lymphocytes, which will have little to no response to LPS stimulation. While there was significant induction of hemoglobin-associated transcripts in chorioamnionitis-exposed monocytes, we believe that red blood cell contamination of our samples is unlikely to be the cause given the purity of our magnetic bead separation and the absence of red blood cells upon microscopic examination of the samples after the separation. We performed ChIP-seq and RNA-seq on total CD14⁺ monocytes rather than monocyte subsets due to limitations in available cell numbers and a recent paper showing no significant difference in monocyte subsets between premature infants with and without chorioamnionitis exposure[7]. We acknowledge that distinct monocyte subsets, including classical (CD14⁺⁺CD16⁻), intermediate (CD14⁺⁺CD16⁺) and nonclassical (CD14⁺CD16⁺) monocytes exhibit unique gene expression profiles and that this may influence the interpretation of our results[47]. Additionally, the inclusion of 2–3 replicates per age/exposure group (n=6–9 total patients) in the ChIP-seq and RNA-seq experiments is low, although this number of replicates has been shown to be sufficient for site discovery in next generation sequencing experiments by the ENCODE consortium[48]. There was a difference in the proportion of infants born by vaginal delivery between healthy preterm and chorioamnionitis-exposed preterm infants. It is possible that mode of delivery rather than chorioamnionitis exposure may have contributed to important differences in monocyte H3K4me3 between these groups as mode of delivery is known to influence neonatal immune function[49]. Additionally, we were unable to control for all potential confounding variables (maternal and neonatal antimicrobial exposure, etc.), which should be taken into account when interpreting our results. We also did not account for other factors regulating mRNA expression, including additional histone modifications (H3K4me1, H3K27me3, etc.), DNA methylation and microRNA expression.

Conclusion

This study reveals that chorioamnionitis exposure remodeled the neonatal monocyte H3K4me3 landscape, with age-inappropriate H3K4me3 patterning. Chorioamnionitis exposure also altered term neonatal monocyte transcription, with a less robust inflammatory response upon a secondary pathogenic exposure. Additionally, H3K4me3 peak presence near a gene was predictive of mRNA expression, irrespective of the genomic location of the

H3K4me3. Together these findings demonstrate that a pathogenic inflammatory exposure in the neonatal period has a profound effect on monocyte transcription and transcriptional regulation that may result in long-term immune dysfunction.

Materials and Methods

Participants

The local Institutional Review Board approved this research protocol and written informed consent was obtained from the parents of all neonatal participants. The study methods conformed to the standards set by the Declaration of Helsinki. Enrolled infants were born at the University of Michigan Medical Center and were classified as term (gestational age 37 weeks or greater), preterm (gestational age 30–36 weeks) or extremely preterm (gestational age under 30 weeks). There is no consensus on what gestational age cutoffs should be used when studying the preterm immune system, and many different cutoff values have been published[17, 50, 51]. We chose the above gestational age cutoffs because as we have published them previously, and believe they provide an immunologically meaningful distinction between groups[17]. Chorioamnionitis was diagnosed upon histopathological examination of the placenta as previously described[52]. Healthy infants had no evidence of inflammation on histopathological examination of the placenta. We enrolled a total of 28 healthy term infants, 27 chorioamnionitis-exposed term infants, 9 healthy preterm infants, 9 chorioamnionitis-exposed preterm infants, 9 healthy extremely preterm infants, and 9 chorioamnionitis-exposed extremely preterm infants.

Blood

Umbilical cord blood was collected from enrolled infants immediately after the delivery of the placenta. The umbilical cord blood was stored in the University of Michigan blood bank at 4°C and the samples were retrieved from the blood bank within 5 days of collection as previously described[17]. The blood samples were diluted 1:2 with sterile 0.9% saline and Ficoll-Isopaque density gradient centrifugation was used to isolate mononuclear cells. Mononuclear cells underwent CD14+ magnetic bead isolation according to the manufacturer's instructions (Miltenyi Biotec, Auburn, CA, USA, 130-050-201). The average purity of the monocytes was 92% by flow cytometry using anti-human CD14 (BioLegend, San Diego, CA, USA, clone HCD14, PerCP/cy5.5). Isolated CD14+ cells were stored in recovery cell culture freezing media (Life Technologies, Carlsbad, CA, USA) in cryovials at –80°C until use. Monocyte viability post freezing was 70–75% by flow cytometry using DAPI (Molecular probes, Eugene, OR).

High Throughput ChIP-Sequencing

ChIP-seq was performed on purified CD14+ monocytes from healthy and chorioamnionitis-exposed term infants, preterm infants, and extremely preterm infants as previously described[17]. Each age group contained 3 biological replicates. In order to obtain approximately 5×10^5 cells per immunoprecipitation, each neonatal sample replicate contained 3 pooled samples (Table 11). The ultrasonication was performed on wet ice using the Branson Digital Sonifier 450 for 240 seconds at 40% amplitude with 0.7 seconds 'on' and 1.3 seconds 'off'. The immunoprecipitation was performed with 4 micrograms of

H3K4me3 antibody (Abcam, Cambridge, MA, USA, ab8580). After library preparation, the samples were pooled in equimolar concentrations and single-end ChIP-seq (50 nt) was performed on an Illumina HiSeq 2500 machine, resulting in 20–100 million reads/sample. The quality of the samples was assessed using FastQC (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>) and ChIPQC[53]. All samples passed quality control measures, which are outlined in Table 12.

ChIP-seq Mapping and Data Analysis

Scythe (<https://github.com/vsbuffalo/scythe>) was used to trim adapter sequences from the raw reads. The reads were then aligned to the *Homo sapiens* genome assembly (Build 37, hg19) with Bowtie2 version 2.0.0 using default parameters[54]. The results were normalized to total read count and library size. MACS2 version 2.1.1.20160309 was used to call peaks by comparing the immunoprecipitated samples to input samples[55]. DiffBind was used to derived consensus peaksets from the biological replicates, and peaks needed to be present in two out of three replicates to be included[56]. The consensus peaksets were used for downstream data analysis. The EdgeR package was used to identify differentially bound sites[20]. The false discovery rate for differentially bound sites was set to <0.1[57, 58]. Peaks were annotated and Gene Ontology terms were identified using ChIPpeakAnno[59]. The UCSC Genome Browser was used for data visualization[18]. Venneuler version 1.1–1 was used construct proportional venn diagrams (<http://www.rforge.net/venneuler/>). The datasets supporting the results are available in the GEO repositories GSE81957 and GSE111873.

Cell culture

Purified CD14+ monocytes were washed twice in sterile RPMI after thawing. The cells were plated at a concentration of 5×10^4 cells/well in 96-well polystyrene cell culture plates in 100 μ l of RPMI media containing 400 mM/L L-glutamine, 10% adult human serum, 1% penicillin/streptomycin, 1% sodium pyruvate and 1% nonessential amino acids. The cells were either left unstimulated or were stimulated with 100 ng/mL lipopolysaccharide (Sigma Aldrich, St. Louis, MO, USA, purified from *E. coli* 055:B5) and were incubated at 37°C with 5% CO₂. Cells were collected for RNA-seq preparation 6 hours after stimulation and for measurement of protein concentration 24 hours after stimulation. Protein levels of the cytokines IL-1 β , IL-6, IL-8, TNF- α and IL-10 were measured from cell culture supernatants using a bead-based multiplex assay (Bio-Rad, Hercules, CA, USA).

RNA-seq

RNA-seq was performed on umbilical cord blood purified CD14+ monocytes from healthy and chorioamnionitis-exposed term neonates in both unstimulated and LPS stimulated conditions. Each exposure/stimulation group contained 3 replicates, except the chorioamnionitis-exposed unstimulated group, which contained 2 replicates as one did not pass quality control. Each replicate contained 3 pooled samples (Table 13). RNA was isolated using an RNeasy micro kit with an on column DNase digestion following the manufacturer's directions (Qiagen, Germantown, MD, USA). cDNA was synthesized and libraries were prepared using the SMARTer stranded total RNA pico kit following the manufacturer's instructions (Takara, Mountain View, CA, USA). The samples were pooled

in equimolar concentrations and paired-end sequencing (50 nt) was performed on an Illumina HiSeq 2500 machine, resulting in 10–20 million reads/sample. The quality of the samples was assessed using FastQC (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>). All samples passed quality control measures, except for replicate 3 of the unstimulated chorioamnionitis-exposed group, which was not sequenced (Table 14).

RNA-seq data analysis

Adapter sequences were trimmed from raw reads using Trimmomatic[60]. Reads were aligned to the *Homo sapiens* genome assembly (Build 37, hg19) with HISAT2 version 2.1.0 using the paired-end option[61]. Subread version 1.6.0 was used to align reads, quantify transcript abundance and assign reads to genomic features[62]. Reads were normalized to total read counts and differentially expressed transcripts were identified using the edgeR package[20]. The false discovery rate for differentially expressed transcripts was set to <0.1[57, 58]. Genes were annotated using MyGene[63]. Pathway analysis was performed using the edgeR package. Network analysis was performed using NetworkAnalyst[64]. The datasets supporting the results are available in the GEO repository GSE111927.

Statistical analysis

Prism 6 was used for basic data analysis. The differences between groups were evaluated with the student's t-test for parametric quantitative data, the Mann-Whitney test for nonparametric quantitative data, ANOVA for multiple comparisons of parametric data and Chi-square test to evaluate relationships between categorical variables. Values of $p < 0.05$ were considered to be significant.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

H3K4me3	trimethylation of lysine 4 of histone 3
ChIP-seq	chromatin immunoprecipitation followed by massively parallel DNA sequencing
RNA-seq	whole transcriptome shotgun sequencing
LPS	lipopolysaccharide
U30	under 30 week extremely preterm healthy infants
U30C	under 30 week extremely preterm chorioamnionitis-exposed infants
O30	over 30 week preterm healthy infants

O30C	over 30 week preterm chorioamnionitis-exposed infants
Term	term healthy infants
TermC	term chorioamnionitis-exposed infants
U	term healthy unstimulated monocytes
L	term healthy LPS stimulated monocytes
CU	term chorioamnionitis-exposed unstimulated monocytes
CL	term chorioamnionitis-exposed LPS stimulated monocytes

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Figure 1: Chorioamnionitis exposure increased term neonatal monocyte H3K4me3 with a preferential gain in introns and intergenic regions.

A) Principal component analysis of neonatal monocyte H3K4me3 normalized read counts. All healthy and chorioamnionitis-exposed preterm monocytes clustered together along principal component 2, with some separation of a few of the chorioamnionitis-exposed monocytes along principal component 1. Term healthy and chorioamnionitis-exposed monocytes had distinct clustering along both principal components 1 and 2, with separation from each other and the preterm monocytes. Chorio=chorioamnionitis exposure. B) Total monocyte H3K4me3 peaks by gestational age and chorioamnionitis exposure. C) Promoter

and exon monocyte H3K4me3 peaks by gestational age and chorioamnionitis exposure. Promoter locations were defined as 1 kb upstream or downstream from transcriptional start sites. D) Intron monocyte H3K4me3 peaks by gestational age and chorioamnionitis exposure. E) Intergenic monocyte H3K4me3 peaks by gestational age and chorioamnionitis exposure. U30(n=3)=under 30 weeks gestation non-chorioamnionitis exposed neonates, U30C(n=3)=under 30 weeks gestation chorioamnionitis exposed neonates, O30(n=3)=30–36 weeks gestation non-chorioamnionitis exposed neonates, O30C(n=3)=30–36 weeks gestation chorioamnionitis exposed neonates, Term(n=3)=37+ weeks gestation non-chorioamnionitis exposed neonates, TermC(n=3)=37+ weeks gestation chorioamnionitis exposed neonates. Box represents mean, error bars are SD. Differences between groups were assessed using the Kruskal-Wallis test with multiple comparisons. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.

Figure 2: Chorioamnionitis stimulated placement of H3K4me3 peaks in gene ontology pathways related to development.

A) Biological gene ontology pathways enriched for H3K4me3 in neonatal monocytes at different gestational ages. The top 10 biological gene ontology pathways enriched for H3K4me3 in chorioamnionitis exposed B) extremely preterm monocytes, C) preterm monocytes and D) term monocytes. U30(n=3)=under 30 weeks gestation non-chorioamnionitis exposed neonates, U30C(n=3)=under 30 weeks gestation chorioamnionitis exposed neonates, O30(n=3)=30–36 weeks gestation non-chorioamnionitis exposed neonates, O30C(n=3)=30–36 weeks gestation chorioamnionitis exposed neonates,

Term(n=3)=37+ weeks gestation non-chorioamnionitis exposed neonates, TermC(n=3)=37+ weeks gestation chorioamnionitis exposed neonates.

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Figure 3: There were varying degrees of shared and unique monocyte H3K4me3 peaks in healthy and chorioamnionitis-exposed monocytes based on gestational age.

Venn diagrams demonstrating unique and common H3K4me3 peaks between healthy and chorioamnionitis-exposed A) extremely preterm monocytes, B) preterm monocytes and C) term monocytes. D) Venn diagram demonstrating common and unique H3K4me3 peaks between chorioamnionitis-exposed monocytes at different gestations. U30=under 30 weeks gestation, O30=30–36 weeks gestation, Term=37+ weeks gestation, Healthy=no chorioamnionitis exposure, Chorio=chorioamnionitis exposure.

Figure 4: Representative common and unique H3K4me3 peaks in term healthy and chorioamnionitis-exposed monocytes.

A) Term chorioamnionitis-exposed and non-exposed monocytes had a common H3K4me3 peak at the promoter site of *FLI1* (Fli-1 proto-oncogene, ETS transcription factor). B) Term healthy monocytes had a unique H3K4me3 peak at the promoter site of *TNFRSF1B* (TNF receptor superfamily member 1B) that was not present in chorioamnionitis-exposed monocytes. C) Term chorioamnionitis-exposed monocytes had multiple unique H3K4me3 peaks inside gene *RFTN2* (Raftlin family member 2) that were not present in unexposed

monocytes. These visual representations were obtained using the UCSC genome browser at <http://genome.ucsc.edu>

A. U30C, O30C and TermC Common H3K4me3 Peak **B. O30C and TermC Common H3K4me3 Peak**

Figure 5: Representative common H3K4me3 peaks in chorioamnionitis-exposed monocytes at different gestations.

A) Chorioamnionitis-exposed monocytes at all gestational ages shared a common H3K4me3 peak inside the gene *TINAGL1* (Tubulointerstitial nephritis antigen like 1). B) preterm and term chorioamnionitis-exposed monocytes shared a common H3K4me3 peak inside the gene *GALNTL6* (Polypeptide N-acetylgalactosaminyltransferase like 6) that was not present in extremely preterm chorioamnionitis-exposed monocytes. These visual representations were obtained using the UCSC genome browser at <http://genome.ucsc.edu>

Figure 6: Term healthy and term chorioamnionitis exposed monocytes had distinctly different H3K4me3 binding affinities.

Heatmap demonstrating affinities for differentially bound H3K4me3 peaks between extremely preterm, preterm and term chorioamnionitis-exposed and unexposed monocytes. The binding affinity of differentially bound H3K4me3 in extremely preterm and preterm healthy and chorioamnionitis-exposed monocytes varied both within gestational age and exposure groups. In contrast, all the replicates within term healthy and term chorioamnionitis-exposed monocytes had similar differentially bound H3K4me3 sites, which were very different between exposure groups. U30(n=3)=under 30 weeks gestation non-chorioamnionitis-exposed neonates, U30C(n=3)=under 30 weeks gestation chorioamnionitis-exposed neonates, O30(n=3)=30–36 weeks gestation non-chorioamnionitis-exposed neonates, O30C(n=3)=30–36 weeks gestation chorioamnionitis-exposed neonates, Term(n=3)=37+ weeks gestation non-chorioamnionitis-exposed neonates, TermC(n=3)=37+ weeks gestation chorioamnionitis-exposed neonates.

A. Increased H3K4me3 Binding Affinity in Term Healthy Monocytes

B. Increased H3K4me3 Binding Affinity in Term Chorioamnionitis-exposed Monocytes

Figure 7: Representative differentially bound H3K4me3 peaks in term healthy and chorioamnionitis-exposed monocytes.

A) Term healthy monocytes had increased H3K4me3 binding affinity at the promoter site of *IL6ST* (Interleukin 6 signal transducer) when compared to term chorioamnionitis-exposed monocytes. B) Term chorioamnionitis-exposed monocytes had increased H3K4me3 binding affinity inside gene *CD84* (Leukocyte differentiation antigen CD84) when compared to term unexposed monocytes. These visual representations were obtained using the UCSC genome browser at <http://genome.ucsc.edu>. Differential binding was determined and p-values were calculated using the DiffBind package.

Figure 8: Chorioamnionitis exposure altered global term neonatal monocyte gene expression both in unstimulated and LPS stimulated conditions.

A) Principal component analysis of RNA-seq normalized read counts using ggplot2 (<http://ggplot.tidyverse.org>). B) Clustered pearson correlation of differentially expressed mRNA transcripts using normalized read counts revealed that unstimulated healthy and chorioamnionitis-exposed term neonatal monocytes had distinct transcript expression profiles. U(n=3)=term healthy unstimulated monocytes, CU(n=2)=term chorioamnionitis-exposed unstimulated monocytes. C) Clustered pearson correlation of differentially expressed mRNA transcripts based on normalized read counts showed that LPS stimulated healthy and chorioamnionitis-exposed monocytes had distinct patterns of RNA expression. L(n=3)=term healthy LPS stimulated monocytes, CL(n=3)=term chorioamnionitis-exposed

LPS stimulated monocytes. Differentially expressed mRNA transcripts were determined using the edgeR package.

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A. Increased Transcripts in LPS Stimulated Healthy Monocytes

KEGG Pathway	Genes in Pathway	p-value	FDR
Cell cycle	63	4.7E-50	1.0E-47
Pathways in cancer	76	9.0E-34	9.8E-32
HTLV-I infection	61	9.6E-33	7.0E-31
Hedgehog signaling pathway	29	2.6E-23	1.1E-21
Wnt signaling pathway	42	1.8E-21	6.3E-21
TGF-beta signaling pathway	31	2.2E-19	6.7E-18
Chronic myeloid leukemia	24	6.5E-14	1.4E-12
Bacterial invasion	20	1.5E-12	3.0E-11
Adherens junction	19	1.2E-9	1.7E-8
P53 signaling pathway	16	2.4E-7	2.5E-6

B. Increased Transcripts in LPS Stimulated Chorioamnionitis-exposed Monocytes

KEGG Pathway	Genes in Pathway	p-value	FDR
Cell cycle	64	7.2E-47	1.6E-44
HTLV-I infection	51	4.7E-20	5.1E-18
Pathways in cancer	56	1.6E-14	1.2E-12
Wnt signaling pathway	32	5.0E-11	2.2E-9
Adherens junction	21	3.5E-10	1.1E-8
Chronic myeloid leukemia	19	3.4E-8	6.6E-7
Gap junction	21	4.1E-8	7.4E-7
Pathogenic Escherichia coli infection	13	5.1E-8	7.9E-7
TGF-beta signaling pathway	20	7.4E-8	1.1E-6
Pyrimidine metabolism	21	4.1E-7	5.3E-6

Figure 9: There is significant overlap in KEGG pathways containing enriched differentially expressed transcripts between LPS stimulated healthy and chorioamnionitis-exposed term neonatal monocytes.

A) Network of differentially expressed transcripts with increased expression in LPS stimulated term healthy monocytes. The top 10 KEGG pathways with enriched transcripts are presented. B) Network of differentially expressed transcripts with increased expression in LPS stimulated term chorioamnionitis-exposed monocytes. The top 10 KEGG pathways containing enriched transcripts are detailed. Network visualization was performed and p-values were derived using NetworkAnalyst.ca.

Figure 10: Term chorioamnionitis-exposed monocytes exhibit decreased pro-inflammatory cytokine expression when a secondary inflammatory stimulus is encountered.

Protein expression of A) IL-1 β , B) IL-6, C) IL-8, D) TNF- α and E) IL-10 were measured in unstimulated and LPS stimulated conditions (100 ng/mL) after 24 hours in culture. Box represents 25th–75th percentile range, whiskers represent minimum to maximum values, line represents median. n=10 healthy term monocytes, n=9 chorioamnionitis-exposed term monocytes, *p<0.05, ***p<0.001, ****p<0.0001. Differences between groups were assessed using the Kruskal-Wallis test with multiple comparisons.

Figure 11: H3K4me3 peak presence near a known gene in neonatal monocytes was a good predictor of active gene transcription.

A) Total consensus H3K4me3 peaks that annotate to known genes in healthy and chorioamnionitis-exposed term neonatal monocytes compared to known annotated mRNA transcripts. mRNA expression was defined as a normalized read count of >10. Consensus H3K4me3 peaks that annotated to known genes compared to quantitative normalized mRNA transcript levels at B) promoter sites, C) introns and D) intergenic sites. U(n=3)=term healthy unstimulated monocytes, CU(n=2)=term chorioamnionitis-exposed unstimulated monocytes, L(n=3)=term healthy LPS stimulated monocytes, CL(n=3)=term chorioamnionitis-exposed LPS stimulated monocytes. Chi-square test was used to evaluate differences in proportions between groups, ****p<0.0001.

Table 1:

ChIP-seq and RNA-seq patient characteristics

Characteristic	ChIP-seq U30 ^a			ChIP-seq O30 ^b		
	U30 ^a	U30C ^{a,d}	p-value	O30 ^b	O30C ^{b,d}	p-value
Total pooled samples (n)	9	9		9	9	
Gestational age (weeks)	27.79 ± 1.98	28.16 ± 1.57	p=0.67	32.54 ± 1.29	33.86 ± 2.43	p=0.17
Birth weight (grams)	1055 ± 414	1177 ± 205	p=0.44	1887 ± 5225	2264 ± 509	p=0.14
Males	5 (56%)	4 (44%)	p=0.64	4 (44%)	6 (67%)	p=0.34
Vaginal delivery	1 (11%)	4 (44%)	p=0.11	1 (11%)	8 (89%)	p=0.001*
Maternal smoking	0 (0%)	1 (11%)	p=0.3	0 (0%)	2 (22%)	p=0.13
Gestational hypertension	2 (22%)	0 (0%)	p=0.13	3 (33%)	1 (11%)	p=0.26
Gestational diabetes	0 (0%)	0 (0%)	p>0.99	0 (0%)	0 (0%)	p>0.99
Prenatal steroids	9 (100%)	9 (100%)	p>0.99	8 (89%)	6 (67%)	p=0.26
	ChIP-seq Term ^c			RNA-seq Term ^c		
	Term ^c	TermC ^{c,d}	p-value	Term ^c	TermC ^{c,d}	p-value
Total pooled samples (n)	9	9		9U ^e 9L ^f	6U ^e 9L ^f	
Gestational age (weeks)	39.9 ± 1.47	40.25 ± 1.08	p=0.57	38.65 ± 1.29 38.65 ± 1.29	39.29 ± 1.31 39.84 ± 1.34	p=0.37 p=0.07
Birth weight (grams)	3779 ± 556	3544 ± 366	p=0.31	3283 ± 623 3283 ± 623	3259 ± 693 3354 ± 580	p=0.94 p=0.81
Males	6 (67%)	6 (67%)	p>0.99	3 (33%) 3 (33%)	3 (50%) 4 (44%)	p=0.52 p=0.63
Vaginal delivery	8 (89%)	4 (44%)	p=0.05	4 (44%) 4 (44%)	3 (50%) 4 (44%)	p=0.83 p>0.99
Maternal smoking	0 (0%)	0 (0%)	p>0.99	0 (0%) 0 (0%)	0 (0%) 0 (0%)	p>0.99 p>0.99
Gestational hypertension	0 (0%)	0 (0%)	p>0.99	0 (0%) 0 (0%)	0 (0%) 1 (11%)	p>0.99 p=0.3
Gestational diabetes	0 (0%)	1 (11%)	p=0.3	0 (0%) 0 (0%)	0 (0%) 1 (11%)	p>0.99 p=0.3
Prenatal steroids	0 (0%)	0 (0%)	p>0.99	0 (0%) 0 (0%)	0 (0%) 0 (0%)	p>0.99 p>0.99

^aU30 = under 30 weeks gestation,^bO30 = 30–36 weeks gestation,^cTerm = term gestation,^dC=chorioamnionitis exposure,^eU=unstimulated,^fL=LPS stimulated. The student's t-test was used to test for differences between quantitative data and the Chi-square test was used to test for differences between categorical data.

Table 2:

Immunologically relevant gene ontology pathways enriched for H3K4me3 in neonatal monocytes

Group	GO Term	Description	Number of genes	p-value
U30C ^{a,d}				
	GO:0050829	Defense response to Gram-negative bacterium	2	0.019
	GO:0006959	Humoral immune response	4	0.024
	GO:0002437	Inflammatory response to antigenic stimulus	2	0.025
	GO:0042742	Defense response to bacterium	4	0.036
	GO:0098542	Defense response to other organism	6	0.037
O30C ^{b,d}				
	GO:0071559	Response to transforming growth factor beta	27	0.011
	GO:0050918	Positive chemotaxis	9	0.018
	GO:0006935	Chemotaxis	68	0.019
	GO:0071634	Regulation of transforming growth factor beta production	5	0.023
	GO:0033081	Regulation of T cell differentiation in thymus	5	0.027
	GO:0048291	Isotype switching to IgG isotypes	3	0.036
	GO:0038123	Toll-like receptor TLR1:TLR2 signaling pathway	10	0.043
TermC ^{c,d}				
	GO:0048291	Isotype switching to IgG isotypes	8	0.0007
	GO:0006935	Chemotaxis	221	0.001
	GO:0002824	Positive regulation of adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains	30	0.003
	GO:0045830	Positive regulation of isotype switching	9	0.004
	GO:0030217	T cell differentiation	68	0.006
	GO:0002714	Positive regulation of B cell mediated immunity	13	0.006
	GO:0002708	Positive regulation of lymphocyte mediated immunity	29	0.007
	GO:0045581	Negative regulation of T cell differentiation	14	0.007
	GO:0033081	Regulation of T cell differentiation in thymus	12	0.008
	GO:0030098	Lymphocyte differentiation	94	0.011
	GO:0002819	Regulation of adaptive immune response	42	0.012
	GO:0002705	Positive regulation of leukocyte mediated immunity	33	0.012
	GO:0043030	Regulation of macrophage activation	12	0.013
	GO:0045954	Positive regulation of natural killer cell mediated cytotoxicity	9	0.02
	GO:0046639	Negative regulation of alpha-beta T cell differentiation	7	0.021
	GO:0046636	Negative regulation of alpha-beta T cell activation	10	0.022
	GO:0032634	Interleukin-5 production	8	0.026
	GO:0045063	T-helper 1 cell differentiation	8	0.026
	GO:0043032	Positive regulation of macrophage activation	6	0.028
	GO:0070664	Negative regulation of leukocyte proliferation	23	0.033

Group	GO Term	Description	Number of genes	p-value
	GO:0002889	Regulation of immunoglobulin mediated immune response	16	0.038
	GO:0050868	Negative regulation of T cell activation	28	0.041
	GO:0050851	Antigen receptor-mediated signaling pathway	49	0.041
	GO:0002717	Positive regulation of natural killer cell mediated immunity	9	0.044
	GO:0032700	Negative regulation of interleukin-17 production	6	0.047
	GO:2000515	Negative regulation of CD4-positive, alpha-beta T cell activation	6	0.047
	GO:0043368	Positive T cell selection	11	0.047
	GO:0050856	Regulation of T cell receptor signaling pathway	11	0.047
	GO:0050853	B cell receptor signaling pathway	16	0.047
	GO:0002706	Regulation of lymphocyte mediated immunity	36	0.048
	GO:0002696	Positive regulation of leukocyte activation	83	0.048

^aU30 = under 30 weeks gestation,

^bO30 = 30–36 weeks gestation,

^cTerm = term gestation,

^dC=chorioamnionitis exposure

Table 3:

Number and location of monocyte consensus H3K4me3 peaks by gestational age and chorioamnionitis exposure

Peak Location	U30 ^a	U30C ^{a,d}	O30 ^b	O30C ^{b,d}	Term ^c	TermC ^{c,d}
Total Consensus Peaks	496	432	374	4254	4250	21,457
Promoter Peaks	4	6	2	26	292	92
Exon Peaks	10	7	6	63	217	429
Intron Peaks	65	67	57	757	646	3436
Intergenic Peaks	417	352	309	3408	3095	17,500

^aU30 = under 30 weeks gestation,

^bO30 = 30–36 weeks gestation,

^cTerm = term gestation,

^dC=chorioamnionitis exposure

Table 4:

Neonatal monocyte H3K4me3 peak width

Age Group	Healthy	Chorioamnionitis-exposed	p-value
U30 ^a	270±240 bp	383±255 bp	0.999
O30 ^b	307±179 bp	142±124 bp	<0.0001
Term ^c	474±364 bp	148±79 bp	<0.0001

^aU30 = under 30 weeks gestation,^bO30 = 30–36 weeks gestation,^cTerm = term gestation, values are mean±SD, p-values calculated using Mann-Whitney test

Table 5:

Differentially bound H3K4me3 peaks

Group 1	Group 2	Total Differentially Bound H3K4me3 Peaks	H3K4me3 Peaks Increased in Group 1	H3K4me3 Peaks Increased in Group 2
U30 ^a	U30C ^{a,d}	1	0	1
O30 ^b	O30C ^{b,d}	68	37	31
Term ^c	TermC ^{c,d}	9633	4387	5246
U30C ^{a,d}	O30C ^{b,d}	60	48	12
U30C ^{a,d}	TermC ^{c,d}	6280	1141	5139
O30C ^{b,d}	TermC ^{c,d}	4600	2975	1625

^aU30 = under 30 weeks gestation,^bO30 = 30–36 weeks gestation,^cTerm = term gestation,^dC=chorioamnionitis exposure

Table 6:

Gene ontology biological pathways enriched for differentially bound H3K4me3

Groups	Increased in Group	GO Term	Term	DB ^e Genes in Pathway	p-value	FDR ^f
U30 ^a vs U30C ^{a,d}	U30	None	None	None	None	None
U30 ^a vs U30C ^{a,d}	U30C	None	None	None	None	None
O30 ^b vs O30C ^{b,d}	O30	None	None	None	None	None
O30 ^b vs O30C ^{b,d}	O30C	None	None	None	None	None
Term ^c vs TermC ^{c,d}	Term	0000209	Protein polyubiquitination	77	3.24E-15	6.22E-12
	Term	0043161	Proteasome-mediated ubiquitin-dependent protein catabolic process	78	4.61E-13	8.91E-12
	Term	0006468	Protein phosphorylation	131	2.47E-10	4.77E-7
	Term	0045893	Positive regulation of transcription, DNA-templated	143	4.63E-10	8.94E-7
	Term	0006357	Regulation of transcription from RNA polymerase II promoter	124	3.24E-9	6.26E-6
	Term	0006915	Apoptotic process	150	6.28E-9	1.21E-5
	Term	0016567	Protein ubiquitination	103	2.56E-8	4.95E-5
	Term	0016032	Viral process	88	8.42E-8	1.63E-4
	Term	0098609	Cell-cell adhesion	81	1.42E-7	2.74E-4
	Term	0002223	Stimulatory C-type lectin receptor signaling pathway	40	4.86E-7	9.39E-4
	Term	0018105	Peptidyl-serine phosphorylation	45	5.37E-7	0.001
	Term	0048010	Vascular endothelial growth factor receptor signaling pathway	31	5.9E-7	0.0011
	Term	0045892	Negative regulation of transcription, DNA-templated	127	9.56E-7	0.0018
	Term	0030036	Actin cytoskeleton organization	45	1.83E-6	0.0035
	Term	0015031	Protein transport	104	2.01E-6	0.0039
	Term	0050852	T cell receptor signaling pathway	49	2.53E-6	0.0049
	Term	0042147	Retrograde transport, endosome to Golgi	29	2.61E-6	0.005
	Term	0007050	Cell cycle arrest	47	3.39E-6	0.0065
	Term	0006914	Autophagy	45	3.64E-6	0.007
	Term	0016569	Covalent chromatin modification	40	4.04E-6	0.0078
	Term	0007049	Cell cycle	64	5.31E-6	0.01
	Term	0006897	Endocytosis	46	5.43E-6	0.01
	Term	0038095	Fc-epsilon receptor signaling pathway	55	6E-6	0.012
	Term	0070936	Protein K48-linked ubiquitination	22	7.68E-6	0.015
	Term	0046777	Protein autophosphorylation	52	2.15E-5	0.042
	Term	0016241	Regulation of macroautophagy	20	3.73E-5	0.072

Groups	Increased in Group	GO Term	Term	DB ^e Genes in Pathway	p-value	FDR ^f
	Term	0043065	Positive regulation of apoptotic process	79	3.75E-5	0.072
	Term	0051301	Cell division	89	4.76E-5	0.092
	Term	0034976	Response to endoplasmic reticulum stress	28	5.11E-5	0.099
Term ^c vs TermC ^{c,d}	TermC	0045944	Positive regulation of transcription from RNA polymerase II promoter	128	7.2E-8	1.35E-4
	TermC	0061337	Cardiac conduction	16	1.39E-6	0.0026
	TermC	0007267	Cell-cell signaling	43	7.61E-6	0.014
U30C ^{a,d} vs O30C ^{b,d}	U30C	None	None	None	None	None
U30C ^{a,d} vs O30C ^{b,d}	O30C	None	None	None	None	None
U30C ^{a,d} vs TermC ^{c,d}	U30C	0000122	Negative regulation of transcription from RNA polymerase II promoter	51	4E-6	0.007
	TermC	0045944	Positive regulation of transcription from RNA polymerase II promoter	119	4.92E-6	0.009
O30C ^{b,d} vs TermC ^{c,d}	O30C	0000209	Protein polyubiquitination	59	4.02E-13	7.62E-10
	O30C	0006351	Transcription, DNA-templated	321	1.69E-11	3.21E-8
	O30C	0043161	Proteasome-mediated ubiquitin-dependent protein catabolic process	58	1.14E-10	2.16E-7
	O30C	0006357	Regulation of transcription from RNA polymerase II promoter	94	6.17E-9	1.17E-5
	O30C	0016032	Viral process	70	1.39E-8	2.64E-5
	O30C	0098609	Cell-cell adhesion	65	1.82E-8	3.45E-5
	O30C	0006915	Apoptotic process	111	3.26E-8	6.18E-5
	O30C	0006468	Protein phosphorylation	94	3.41E-8	6.46E-5
	O30C	0002223	Stimulatory C-type lectin receptor signaling pathway	34	4.87E-8	9.23E-5
	O30C	0070936	Protein K48-linked ubiquitination	21	7.97E-8	1.51E-4
	O30C	0045944	Positive regulation of transcription from RNA polymerase II promoter	164	1.04E-6	0.002
	O30C	0038095	Fc-epsilon receptor signaling pathway	44	2.02E-6	0.0038
	O30C	0050852	T cell receptor signaling pathway	38	4.37E-6	0.008
	O30C	0000122	Negative regulation of transcription from RNA polymerase II promoter	124	6.06E-6	0.011
	O30C	0007049	Cell cycle	49	7.43E-6	0.014
	O30C	0042147	Retrograde transport, endosome to Golgi	22	2.24E-5	0.042
	O30C	0007050	Cell cycle arrest	35	2.39E-5	0.045
	O30C	0030036	Actin cytoskeleton organization	33	2.64E-5	0.05
	O30C	0051403	Stress-activated MAPK cascade	11	4.61E-5	0.087
O30C ^{b,d} vs TermC ^{c,d}	TermC	None	None	None	None	None

^aU30 = under 30 weeks gestation,

b_{O30} = 30–36 weeks gestation,

c_{Term} = term gestation,

d_C = chorioamnionitis exposure,

e_{DB} = differentially bound,

f_{FDR} = false discovery rate

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Table 7:

Differentially expressed mRNA transcripts

Group 1	Group 2	Total Differentially Expressed Transcripts	Transcripts Increased in Group 1	Transcripts Increased in Group 2
U ^a	L ^b	516	266	250
CU ^c	CL ^d	304	223	81
U ^a	CU ^c	135	23	112
L ^b	CL ^d	594	263	331

^aU=term healthy unstimulated,^bL=term healthy LPS stimulated,^cCU=term chorioamnionitis-exposed unstimulated,^dCL=term chorioamnionitis-exposed LPS stimulated

Table 8:

Gene ontology pathways enriched for differentially expressed transcripts

Groups	Increased in Group	GO Term	Term	Ontology	DE ^e Genes in Pathway	p-value	Benjamini
U ^a vs L ^b							
	U	0003676	Nucleic acid binding	MF	29	8.3E-6	2.8E-3
	U	0046872	Metal ion binding	MF	45	3.4E-5	5.6E-3
	L	0005615	Extracellular space	CC	32	1.3E-5	2.6E-03
	L	0005125	Cytokine activity	MF	10	6.5E-5	2.0E-02
	L	0006954	Inflammatory response	BP	15	3.7E-5	4E-2
CU ^c vs CL ^d							
	U	None	None	None	None	None	None
	L	None	None	None	None	None	None
U ^a vs CU ^c							
	U	None	None	None	None	None	None
	CU	0015671	Oxygen transport	BP	6	7.1E-9	3.3E-6
	CU	0005344	Oxygen transporter activity	MF	6	3.8E-9	6E-7
	CU	0015701	Bicarbonate transport	BP	7	6.9E-8	1.6E-05
L ^b vs CL ^d							
	L	None	None	None	None	None	None
	CL	0005344	Oxygen transporter activity	MF	8	3.9E-10	1.4E-07
	CL	0015671	Oxygen transport	BP	8	7.5E-10	8.6E-07
	CL	0019825	Oxygen binding	MF	9	3.4E-7	6.2E-05
	CL	0005506	Iron ion binding	MF	11	8.1E-5	9.7E-03

^aU=term healthy unstimulated monocytes,^bL=term healthy LPS stimulated monocytes,^cU=term chorioamnionitis-exposed unstimulated monocytes,^dCL=term chorioamnionitis-exposed LPS stimulated monocytes,^eDE=differentially expressed

Table 9:

Comparison of differentially bound H3K4me3 and differentially expressed mRNA transcripts

Increased Differentially Bound H3K4me3	Increased Differential mRNA Transcripts	Comparison Group	Total Non-duplicated Differentially Bound H3K4me3	Total Differential mRNA Transcripts	Shared Differential H3K4me3 and mRNA Transcripts
Healthy ^a	U ^c	CU ^e	3386	23	1
Healthy ^a	L ^d	CL ^f	3386	263	23
Chorio ^b	CU ^e	U ^c	1930	112	9
Chorio ^b	CL ^f	L ^d	1930	331	31

^aHealthy=term healthy monocytes,^bChorio=term chorioamnionitis-exposed monocytes,^cU=term healthy unstimulated,^dL=term healthy LPS stimulated,^eCU=term chorioamnionitis-exposed unstimulated,^fCL=term chorioamnionitis-exposed LPS stimulated

Table 10:

Locations of shared differentially bound H3K4me3 and differentially expressed mRNA

Increased Differentially Bound H3K4me3	Increased Differential mRNA Transcripts	Comparison Group	Gene	H3K4me3 Peak Location	Differentially Bound H3K4me3 p-value	Differentially Expressed mRNA Transcript p-value
Healthy ^a	U ^c	CU ^e	<i>STXBP5-AS1</i>	Promoter	2.58E-21	4E-4
Healthy ^a	L ^d	CL ^f	<i>ITGB3BP</i>	Promoter	3.07E-31	0.0019
			<i>TPGS1</i>	Promoter	2.28E-29	6.5E-4
			<i>TACSTD2</i>	Inside gene	1.06E-26	0.0016
			<i>GPSM2</i>	Inside gene	2.19E-24	0.0013
			<i>LRR1</i>	Promoter	8.4E-24	0.0025
			<i>RFNG</i>	Promoter	1.6E-23	0.0035
			<i>PIGZ</i>	Upstream	2.59E-23	2.5E-4
			<i>PGM2</i>	Inside gene	2.76E-23	0.001
			<i>RGS9BP</i>	Promoter	2.87E-23	2E-4
			<i>FRS3</i>	Upstream	3.24E-19	2E-4
			<i>NAA50</i>	Inside gene	6.06E-19	0.0034
			<i>ZNF747</i>	Promoter	2.15E-18	5.2E-4
			<i>IMPACT</i>	Inside gene	3.95E-18	0.0034
			<i>FKBP9</i>	Promoter	2.21E-17	4.3E-5
			<i>PRRG4</i>	Promoter	4.34E-17	1.3E-5
			<i>NDC1</i>	Inside gene	4.63E-17	0.0028
			<i>PKIB</i>	Upstream	6.33E-17	1.2E-5
			<i>RALGAPA2</i>	Promoter	8.71E-16	9.9E-5
			<i>B4GALT6</i>	Upstream	6.67E-15	0.0041
			<i>SNX33</i>	Promoter	6.68E-15	2.3E-4
			<i>KCTD3</i>	Promoter	4.24E-13	2.5E-4
			<i>TTC12</i>	Promoter	5.72E-12	3E-5
			<i>MAD2L1</i>	Promoter	1.63E-11	7.2E-5
Chorio ^b	CU ^e	U ^c	<i>RASEF</i>	Downstream	1.1E-6	1.4E-4
			<i>LRRC70</i>	Downstream	9.4E-6	5.7E-4
			<i>KLHDC9</i>	Upstream	1.3E-4	8.7E-5
			<i>CFAP46</i>	Inside gene	9E-4	1.6E-4
			<i>KEL</i>	Upstream	0.0017	1.6E-4
			<i>SARDH</i>	Upstream	0.0025	3.7E-5
			<i>ZNF608</i>	Downstream	0.0026	3.6E-5
			<i>CLEC1B</i>	Upstream	0.0045	1.7E-4
			<i>MAP9</i>	Upstream	0.015	2.6E-4

Increased Differentially Bound H3K4me3	Increased Differential mRNA Transcripts	Comparison Group	Gene	H3K4me3 Peak Location	Differentially Bound H3K4me3 p-value	Differentially Expressed mRNA Transcript p-value
Chorio ^b	CL ^f	L ^d	<i>LRGUK</i>	Inside gene	4.1E-9	0.0017
			<i>DNAJC5B</i>	Upstream	3.6E-8	4E-4
			<i>HIBCH</i>	Upstream	2E-6	0.0042
			<i>FOXP3</i>	Inside gene	3.1E-6	5.5E-4
			<i>ALAS2</i>	Inside gene	2.7E-5	0.002
			<i>MECOM</i>	Inside gene	2.8E-5	6.9E-4
			<i>ARV1</i>	Inside gene	3.9E-5	0.0014
			<i>KLHDC9</i>	Upstream	1.3E-4	3.9E-5
			<i>MRPL45P2</i>	Downstream	2E-4	1.8E-4
			<i>TRUB1</i>	Upstream	2.2E-4	7E-5
			<i>CACNA2D2</i>	Inside gene	2.3E-4	5.3E-4
			<i>EXTL2</i>	Downstream	4.8E-4	0.0031
			<i>LGR5</i>	Upstream	8.9E-4	0.0014
			<i>NMNAT2</i>	Inside gene	0.0011	0.0022
			<i>MIR652</i>	Downstream	0.0013	8.7E-4
			<i>DMBX1</i>	Upstream	0.0025	1.3E-4
			<i>DAPK2</i>	Inside gene	0.0033	5.5E-5
			<i>RASGRF1</i>	Inside gene	0.0035	4.1E-5
			<i>CEP44</i>	Upstream	0.0036	0.0015
			<i>CETN3</i>	Downstream	0.0043	0.0023
			<i>KCNIP4</i>	Inside gene	0.005	1.4E-4
			<i>CMKLR1</i>	Upstream	0.0055	2.4E-5
			<i>MDGA1</i>	Promoter	0.01	0.0023
			<i>TTYH1</i>	Upstream	0.01	0.0022
			<i>DYNC2H1</i>	Inside gene	0.014	6.8E-8
			<i>AXIN2</i>	Downstream	0.014	2.9E-4
			<i>ZNF385C</i>	Upstream	0.022	0.0011
			<i>NCAPH</i>	Downstream	0.024	1E-5
			<i>DET1</i>	Upstream	0.035	0.0024
			<i>VIL1</i>	Upstream	0.035	0.0038
			<i>ACADSB</i>	Downstream	0.036	2.2E-4

^aHealthy=term healthy monocytes,

^bChorio=term chorioamnionitis-exposed monocytes,

^cU=term healthy unstimulated,

^dL=term healthy LPS stimulated,

^eCU=term chorioamnionitis-exposed unstimulated,

$f_{\text{CL=term chorioamnionitis-exposed LPS stimulated}}$

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Table 11:

ChIP-seq patient sample composition

Group	Replicate	Age (weeks gestation) and Sex	# of Cells used in ChIP
U301	1	24M, 24.86F, 29.14M	8.8×10^5
U302	2	28.14F, 29.14F, 29.29M	8.5×10^5
U303	3	28.14M, 28.14F, 29.29M	8.2×10^5
U30C1	1	29.71M, 28F, 29M	8.5×10^5
U30C2	2	29.71M, 28.43M, 28.71F	2.2×10^6
U30C3	3	25.86F, 25.29F, 28.71F	7×10^5
O301	1	30.71M, 31.43F, 31.43F	7.0×10^5
O302	2	32.43M, 33.43M, 34.29F	5.5×10^5
O303	3	31.71M, 33.43F, 34F	6.0×10^5
O30C1	1	34.43M, 34M, 34.71F	1.4×10^6
O30C2	2	30M, 36.71F, 35.57M	1×10^6
O30C3	3	30.86M, 31.86F, 36.57M	4.8×10^5
Term1	1	37.14F, 38M, 41M	5.0×10^5
Term2	2	39.86F, 41M, 41.14M	5.5×10^5
Term3	3	39.43M, 40.43F, 41.14M	6.0×10^5
TermC1	1	40.14M, 41.29F, 40.86M	3.6×10^5
TermC2	2	37.86M, 39.71M, 40.71F	4.7×10^5
TermC3	3	39.86F, 41.43M, 40.43M	3.9×10^5

U30 = under 30 weeks gestation, O30 = 30–36 weeks gestation, Term = term gestation, C=chorioamnionitis exposure, M=male, F=female

Table 12:

H3K4me3 ChIP-seq quality control data

Sample	Sequencing Depth (reads)	Read Quality (Phred Score ^a)	Uniquely Mapping Reads	Reads Mapped (%)	SSD ^b	FRIP ^c
U301	57,276,495	39	72,464	100	14.3	22.8
U302	49,342,352	39	72,665	100	11.6	18.7
U303	29,802,440	39	291,617	100	4.07	5.89
U30C1	70,437,662	38.4	396,500	100	6.71	7.9
U30C2	50,711,822	38.3	134,943	100	8.75	12.5
U30C3	43,468,108	38.3	379,625	100	5.02	7.14
O301	39,347,378	39	87,324	100	7.98	12
O302	62,638,230	39	310,426	100	6.87	7.01
O303	44,318,249	39	215,790	100	5.52	6.55
O30C1	49,479,468	38.4	983,885	100	4.85	5.22
O30C2	103,309,230	36.6	208,965	100	3.66	6.46
O30C3	56,772,587	36.6	111,612	100	3.08	6.85
Term1	71,263,489	39	252,327	100	12.8	14.8
Term2	72,201,221	39	251,160	100	11.6	14
Term3	60,628,889	39	145,300	100	14.2	18.9
TermC1	72,842,732	36.4	96,237	100	6.37	5.29
TermC2	82,604,593	36.4	104,332	100	12.2	10.6
TermC3	79,096,520	36.6	126,490	100	4.83	5.81

^aPhred scores greater than 30 are considered excellent, with a base call accuracy of over 99.9%.

^bSSD is the standard deviation of signal pile-up along the genome normalized to the total number of reads; values greater than 2 indicate very good chromatin immunoprecipitation enrichment.

^cFRIP is the percentage of reads that overlap peaks; values greater than 5 indicate successful chromatin immunoprecipitation enrichment.

Table 13:

RNA-seq patient sample composition

Group	Replicate	Age (weeks gestation) and Sex	# of Cells used in RNA-seq
U1	1	37F, 39.57M, 37.86F	1.7×10^5
U2	2	40.43F, 38.86M, 39.14F	1.8×10^5
U3	3	37.86F, 40.14M, 37F	1.6×10^5
L1	1	37F, 39.57M, 37.86F	1.7×10^5
L2	2	40.43F, 38.86M, 39.14F	1.8×10^5
L3	3	37.86F, 40.14M, 37F	1.6×10^5
CU1	1	38.14F, 41F, 40.43M	1.3×10^5
CU2	2	39.86F, 38.14M, 38.14M	1.6×10^5
CU3	3	41.14F, 41.14M, 40.57F	1.7×10^5
CL1	1	38.14F, 41F, 40.43M	1.3×10^5
CL2	2	39.86F, 38.14M, 38.14M	1.6×10^5
CL3	3	41.14F, 41.14M, 40.57F	1.7×10^5

U=healthy unstimulated monocytes, L=healthy LPS stimulated monocytes, CU=chorioamnionitis-exposed unstimulated monocytes, CL=chorioamnionitis-exposed LPS stimulated monocytes, M=male, F=female

Table 14:

RNA-seq quality control data

Sample	Sequencing Depth (reads)	Read Quality (Phred Score ^a)	RNA Concentration (nanograms)	RIN ^b
U1	13,325,665	35.8	26.4	8.9
U2	14,003,586	35.6	43.5	9.2
U3	16,590,521	35.6	13.1	1.1
L1	14,090,634	35.8	11.3	8.6
L2	16,437,185	35.8	36.9	8.7
L3	14,342,866	35.8	5.7	8.6
CU1	14,797,385	35.8	46.5	9.0
CU2	14,179,302	35.8	45.9	6.8
CU3	Not sequenced	Not sequenced	<5	<1
CL1	15,336,848	35.7	13	6.5
CL2	11,726,579	35.7	27.4	8.8
CL3	14,963,332	35.6	16.1	8.2

^aPhred scores greater than 30 are considered excellent, with a base call accuracy of over 99.9%.

^bRNA integrity number representing the degree of RNA degradation; values below 7 indicate some degree of degradation. While a RIN value of greater than 7 is recommended to proceed with RNA-seq, accurate transcript quantification can be provided by degraded RNA samples[65].