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REVIEW ARTICLE

Epigenetic regulation of pediatric and neonatal immune responses

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ABSTRACT: Epigenetic regulation of transcription is a collective term that refers to mechanisms known to regulate gene transcription without changing the underlying DNA sequence. These mechanisms include DNA methylation and histone tail modifications which influence chromatin accessibility, and microRNAs that act through post-transcriptional gene silencing. Epigenetics is known to regulate a variety of biological processes, and the role of epigtenetics in immunity and immune-mediated diseases is becoming increasingly recognized. While DNA methylation is the most widely studied, each of these systems play an important role in the development and maintenance of appropriate immune responses. There is clear evidence that epigenetic mechanisms contribute to developmental stage-specific immune responses in a cell-specific manner. There is also mounting evidence that prenatal exposures alter epigenetic profiles and subsequent immune function in exposed offspring. Early life exposures that are associated with poor long-term health outcomes also appear to impact immune specific epigenetic patterning. Finally, each of these epigenetic mechanisms contribute to the pathogenesis of a wide variety of diseases that manifest during childhood. This review will discuss each of these areas in detail.

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IMPACT:

- Epigenetics, including DNA methylation, histone tail modifications, and microRNA expression, dictate immune cell phenotypes.
- Epigenetics influence immune development and subsequent immune health.
- Prenatal, perinatal, and postnatal exposures alter immune cell epigenetic profiles and subsequent immune function.
- Numerous pediatric-onset diseases have an epigenetic component.
- Several successful strategies for childhood diseases target epigenetic mechanisms.

INTRODUCTION

Epigenetics refers to heritable changes in phenotype that do not alter the underlying genetic code. Classical epigenetics refers to DNA methylation and histone tail modifications, both of which influence chromatin accessibility and determine whether transcription factors are able to access gene promoters and initiate transcription. Nonclassical epigenetics typically refers to microRNAs, which are involved in post-transcriptional regulation of gene expression. DNA methylation involves methylation of the fifth carbon of cytosines (5-methylcytosine), which are principally located in CpG dinucleotides.[1] DNA methylation is associated with transcriptional repression and is necessary for embryonic development, genomic imprinting, and X chromosome inactivation.[2] In contrast to DNA methylation, which modifies the chemistry of nucleic acids, histone tail modifications alter the conformation of the proteins that enable DNA to fit inside the nucleus. DNA wraps around histones to form secondary and tertiary structures that pack the DNA into the classical chromosome shapes observed in karyotyping assays. Post-translational modification of histone tails determines whether the surrounding DNA is compact or open. This is a critical function as active gene transcription requires an open chromatin state.[3] Histone tail modifications include methylation, acetylation, phosphorylation, ubiquitylation, SUMOylation, glycosylation, and ADP-ribosylation, although methylation and acetylation are the two most commonly studied.[4] Histone tail modifications are crucial for basic cell function during embryonic development and following birth.[4]

The production of microRNAs is a separate process cells employ for transcriptional regulation. MicroRNAs are small non-coding RNAs that range from 19 to 25 nucleotides in length.[5] The sequence of these RNAs is usually the reverse complement of a messenger RNA (mRNA) that is actively transcribed by the cell. When a microRNA binds to its target mRNA, the post-transcriptional processing of that mRNA is altered. The majority of microRNAs suppress expression of their target mRNA and target it for degradation, although there are reports of microRNAs facilitating increased mRNA expression.[6,7] MicroRNAs are associated with many developmental processes and have been proposed as biomarkers in many disease states.[8,9] Figure 1 provides a visual representation of these different epigenetic mechanisms.

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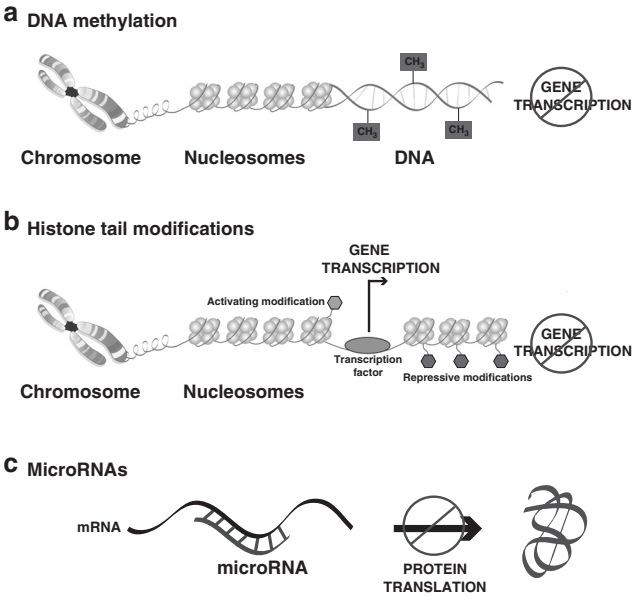

Fig. 1 Epigenetic regulation of gene expression. a The additional of methyl groups to the DNA backbone results in repression of gene transcription. **b** The addition of certain activating modifications to histone tails (H3K4me3, H3K36me3, H3K9ac, H3K18ac among others) results in a relaxed chromatin configuration and active gene transcription, while the addition of other repressive modifications to histone tails (H3K9me3, H3K27me3, etc.) results in a condensed chromatin configuration and reduction in gene transcription. **c** MicroRNAs bind to messenger RNAs and inhibit protein translation.

The study of epigenetics has increased dramatically over the past 20 years. DNA methylation, histone tail modifications, and microRNA expression often work in concert to regulate gene expression, but are often studied separately. Each of these epigenetic mechanisms have been shown to regulate gene expression in a wide variety of biological processes, including embryonic development, cancer, metabolism, and immunity.[2,4,10–12] In this review we will summarize the current literature on the role of these different epigenetic processes in pediatric and neonatal immunity and immune-mediated diseases. An outline of the topics covered in this review article is provided in Fig. 2.

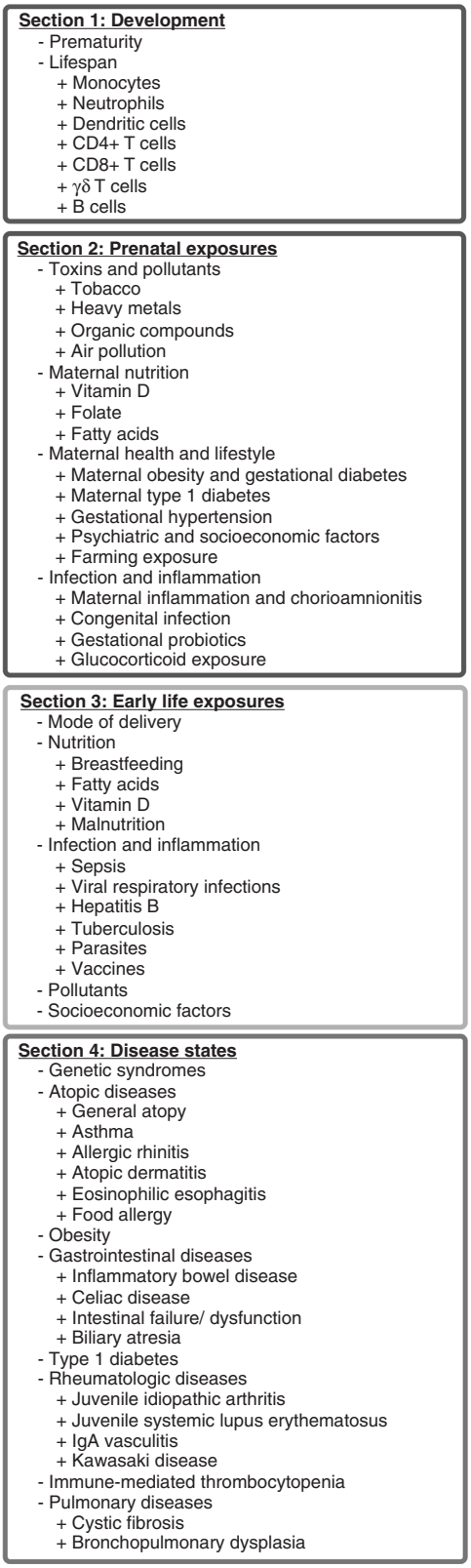

Fig. 2 Article table of contents by section. Created with BioRender.com.

DEVELOPMENT

Prematurity

Premature neonates have an increased risk of infection compared to term neonates, and this is often attributed to immune system immaturity.[13,14] Umbilical cord blood cells from preterm neonates demonstrate differential DNA methylation compared to term neonates, and these differentially methylated sites are enriched in pathways involved in fetal development and immune responses.[15–22] DNA methylation patterns in whole umbilical cord blood correspond well to ultrasound-predicted gestational age, and have been suggested as a reliable method of estimating gestational age when dating is uncertain.[23] While most of these studies compare either whole umbilical cord blood or isolated cord blood mononuclear cells, many differences appear to be cell specific.[18] Nucleated red blood cells, which comprise up to 10% of umbilical cord blood, demonstrate the most differentially methylated sites between preterm and term neonates (9258 sites).[18,24] The majority of these sites are hypomethylated in term neonates.[18] Umbilical cord blood immune subsets have significantly less differential methylation, with under 1000 differentially methylated sites noted in T cells, monocytes, and granulocytes between preterm and term neonates.[18] Compared to preterm neonates, global hypermethylation is noted in term T cells, with global hypomethylation in term monocytes and granulocytes.[18] The methylation patterns in term immune cell subsets are consistent with terminally differentiated and functional immune

cells.[25–27] Many of the gestational-age associated differences in DNA methylation persist during early childhood but resolve by adolescence.[16,19,20] Very little is known about histone tail modifications and microRNA expression in preterm neonates. One study found that umbilical cord blood mononuclear cells from term neonates have more of the activating histone modification H3K4me3 at promoter sites of the pro-inflammatory cytokines *IL1B*, *IL6*, *IL12B*, and *TNF* compared to preterm neonates.[28] No differences were observed in the repressive modification H3K27me3 at these same sites.[28] Taken together, these results suggest that term immune cells have greater epigenetic "maturity" than preterm cells, which may play a role in infection susceptibility.

Lifespan

Immune responses and infection risk differ across the lifespan. Neonates and infants have altered inflammatory responses and an increased risk of invasive bacterial infection, many of which are easily cleared by older children and adults.[29] Evidence is accumulating that epigenetics contributes to these differences. Whole blood demonstrates developmental-stage-specific differences in DNA methylation. Umbilical cord blood is hypermethylated compared to peripheral blood from infants, children, and adolescents.[16,30–33] Over 50% of the methylated CpG sites present in umbilical cord blood demonstrate change over time, with most of these locations undergoing demethylation as age advances.[33] Sites that become hypomethylated (more accessible) with age are enriched in immune and inflammatory pathways, while sites that gain methylation (less accessible) with age are enriched in developmental pathways.[16,30–33] Interestingly, low birth weight term neonates have differential umbilical cord blood DNA methylation compared to normal birth weight term neonates.[34] These differences are present in immune-related pathways, and may contribute to the altered immune function seen in small for gestational age neonates.[35] Isolated mononuclear cells also undergo age-related changes in DNA methylation.[36–40] Studies are conflicting about whether neonatal mononuclear cells demonstrate global hypermethylation,[37,38] hypomethylation,[40] or equivalent methylation[39] compared to other age groups. The studies do agree that mononuclear cells lose methylation in immune pathways while they gain methylation in developmental pathways as age progresses, similar to whole blood.[36,37,39] Puberty is a period of accelerated sex-specific DNA methylation changes in mononuclear cells.[41] Many of the differentially methylated sites in post-pubertal females map to immune and reproductive hormone signaling pathways, while those in post-pubertal males map to adrenaline biosynthesis pathways. These results may contribute to sex-specific differences in immune-mediated diseases seen in adulthood.[41] Neonatal mononuclear cells also have differential expression of immunomodulatory microRNAs compared to cells from 7-year-old children. The majority of these microRNAs are downregulated in neonatal mononuclear cells (let-7e-5p, miR-19a-3p, miR-200a, miR-142-5p, miR-146a-5p, let-7c-5p, miR-301a-3p, and let-7d-5p).[42] miR-150-5p is the lone upregulated microRNA in neonatal mononuclear cells.[42] Additionally, there is a gain of the activating histone modification H3K4me3 and the repressive histone modification H3K9me3 at the promoter sites of the pro-inflammatory cytokines *IL1B*, *IL6*, and *TNF* over the first 6 weeks of life in neonatal mononuclear cells.[43] These results provide convincing evidence that immune cells undergo age-related epigenetic changes that contribute to developmental stage-specific immune responses.

Similar to findings in preterm neonates, immune cell subpopulations demonstrate global but disparate age-related changes in DNA methylation, histone tail modifications, and microRNA expression.[44–49] DNA methylation at several immunologically relevant genes, including *TNF*, *KIR2DL4*, *IFNG*, *IL4*, and *IL8*, varies significantly between total mononuclear cells and immune cell subpopulations.[38] This suggests that unsorted mononuclear cells are not a good representative model for DNA methylation patterns in immune cell subpopulations. Age-related epigenetic changes for different immune subpopulations will be discussed next.

Monocytes. Monocytes are the precursor of several innate immune cell populations, including macrophages and dendritic cells. Each of these cell types perform critical immune functions in both neonates and adults, including cytokine production, antigen processing and presentation and bacterial elimination. Neonatal monocytes are less inflammatory than their adult counterparts, and epigenetics is thought to contribute to this. Neonatal monocytes and fetal placental macrophages show DNA hypermethylation near several immune response genes compared to monocytes and decidual macrophages from the mother, including *ADA*, *PGLYRP1*, *TRAF1*, *IL1B*, *PTGDR*, *LAG3*, and *CD79A*.[50] These findings are proposed to contribute to the anti-inflammatory phenotype of monocytes and macrophages at the feto-maternal interface. Additionally, monocytes from children demonstrate global DNA hypomethylation compared to adult monocytes.[45] Many differentially methylated sites include immune genes, and these differences are associated with increased expression of IL-8, IL-10, and IL-12p70 in adult monocytes following TLR4 or TLR1/2 stimulation.[45] Neonatal monocytes also have differential expression of several microRNAs compared to adult monocytes following lipopolysaccharide (LPS) stimulation.[48,51,52] Neonatal monocytes have enhanced LPS-induced expression of miR-146a, miR-18a, and miR-155 compared to adults, and this is thought to negatively regulate TLR4 signaling and contribute to decreased inflammatory responses in neonatal monocytes.[49,51] Somewhat contrary to this, neonatal monocytes have more pronounced downregulation of miR-103, miR-125b, miR-130a, miR-454-3p, and miR-542-3p compared to adults following LPS-stimulation, which is thought to contribute to increased neonatal monocyte tumor necrosis factor-α (TNF-α) expression.[52] Genome-wide histone tail modification profiling reveals that neonatal monocytes have a global increase in the enhancer modification H3K4me1, a global decrease in the activating modification H3K4me3 and no difference in the enhancer modification H3K27ac, the activating modification H3K36me3 or the repressive modifications H3K9me3 and H3K27me3 compared to adults.[28] The age-related gain in H3K4me3 is primarily in promoter locations, and several immune-related genes show increased promoter-site H3K4me3 in adult monocytes. Increased H3K4me3 at the promoter sites of *IL1B*, *TNF*, *CCR2*, *CD300C*, and *ILF2* are associated with increased IL-1β, TNF-α, CCR2, CD300C, and *ILF2* expression in adult monocytes.[28] These studies suggest that epigenetics contributes to developmental stage-specific differences in monocyte responses.

Neutrophils. Neutrophils are short-lived innate immune cell that are important for the elimination of bacteria and fungi. As with monocytes, neonatal neutrophils are less inflammatory than those found in adults. Neonatal neutrophils have decreased LPS-induced miR-142 and let-7g expression compared to adults.[53] Both miR-142 and let-7g repress IL-6 expression, and lower expression in neonatal neutrophils is associated with increased IL-6 expression compared to adults.[53] Cows also demonstrate an age-related increase in neutrophil miR-125b, miR-146a, miR-155, and miR-9 expression, which is associated with a more robust pro-inflammatory response over time.[54] Additionally, neutrophils from neonatal foals have a reduction in the activating histone tail modification H3K4me3 without a difference in the repressive modification H3K27me3 at immunologically relevant promoters compared to older foals.[55] These differences are related to deficient neonatal neutrophil responses, including poor reactive oxygen species generation and diminished IFN-γ expression.[55]

Dendritic cells. Very little is known about age-related epigenetic changes in dendritic cells. There is a single study showing that neonatal plasmacytoid dendritic cells have increased miR-146a and miR-155 expression compared to adults.[56] These findings are thought to contribute to dampened TLR9-induced IFN-α production and a less inflammatory phenotype in neonatal dendritic cells.[56]

CD4+ T cells. CD4+ T cells are adaptive immune cells that work with other cell types, including macrophages, B cells, and CD8+ T cells, to generate long-lasting immunity. Seminal studies regarding age-related DNA methylation changes in CD4+ T cells were performed in mice. Hypomethylation of the Th2 locus (*CNS-1*, *IL13*, *IL4*, *CIRE*) and hypermethylation of the Treg locus *FOXP3* and Th1 locus *IFNG* was noted in neonatal CD4+ T cells.[57,58] These differences were associated with increased expression of the Th2 cytokines IL-4 and IL-13 in neonatal cells, leading to a Th2 rather than a Th1 phenotype.[58] Some of these findings have been replicated in human studies. Human neonatal CD4+ T cells demonstrate differences in global DNA methylation compared to cells from children and adults.[44,59] Neonatal cells show global hypomethylation compared to cells from 12-month-old infants[59] but global hypermethylation compared to cells from adults.[44] Human neonatal CD4+ T cells have hypermethylation of the Th1 locus *IFNG*, the Th17 locus *IL17*, and the Treg locus *FOXP3* compared to cells from infants, children, and adults.[60,61] However, human neonatal cells show either hypermethylation (*IL13*) or equivalent methylation (*IL4*) at Th2 loci compared to infants, children, and adults.[60,61] Differences in microRNA expression also contribute to the Th2 bias seen in neonatal CD4+ T cells.[44,48,62,63] Neonatal cells have increased miR-184 and miR-34c-5p and decreased let-7b-5p and let-7c expression compared to adults.[48,49,62] These findings are associated with decreased IL-2 expression and increased IL-10 and IL-13 expression in neonatal cells.[48,49,62] Neonatal CD4+ T cells also have higher miR-181a expression compared to adult cells, which contributes to increased activation-induced calcium flux in the neonatal cells.[63] These findings do not translate to increased neonatal cytokine expression, as calcium flux is decoupled from downstream NFAT/AP-1 induction in neonatal cells, which is required for activation-induced cytokine expression.[63] Neonatal CD4+ T cells demonstrate an increase in the repressive histone tail modification H3K27me3 with equivalent levels of the activating modifications H3K4me3 and H3 global acetylation at the promoter site of the Th9 transcription factor *PU.I* compared to adult cells.[64] These differences relate to a failure of neonatal cells to differentiate into Th9 cells under conventional Th9-inducing conditions.[64] Taken together, these findings provide mechanistic insight into the maintenance of age-related CD4+ T cell phenotypes.

CD8+ T cells. CD8+ T cells are important for the elimination of viruses and intracellular bacterial infections. Neonatal and adult CD8+ T cells express equivalent levels of IFN-γ, and this is associated with the similar levels of DNA methylation at the *IFNG* promoter.[61] Neonatal CD8+ T cells have lower expression of the microRNAs let-7b-5p and let-7c compared to adult cells.[48] This is thought to explain the increased proliferative capacity of neonatal CD8+ T cells, as decreased let-7 expression enhances clonal CD8+ T cell expansion.[65,66] Similarly, neonatal CD8+ T cells have decreased miR-29 expression compared to adults.[67] This is proposed to contribute to the reduced ability of neonatal cells to generate memory cells during infection as decreased miR-29 is associated with a bias toward cell activation and differentiation into effector cells rather than generation of memory cells.[67] Neonatal and adult CD8+ T cells also exhibit global differences in histone modifications. Adult cells demonstrate an increase in the activating modification H3K4me3 and the enhancer modification H3K27ac and a decrease in the repressive modification H3K27me3 at loci of highly expressed genes compared to neonatal cells.[68] These findings are associated with reduced cytotoxicity in neonatal cells.[68]

γδ T cells. Gamma–delta T cells (γδ T cells) comprise a small subset of T cells in humans with a limited T cell repertoire. They are important in many aspects of mucosal immunity, including gut immune homeostasis. PD1 is a negative regulator of T cell receptor signaling, and plays an important role in maintaining immune tolerance at the feto-maternal interface during pregnancy.[69–71] Neonatal Vδ2T lymphocytes, a subset of γδ T lymphocytes, demonstrate decreased DNA methylation at the *PD1* locus and increased *PD1* expression compared to adults.[72] This suggests that neonatal Vδ2 T lymphocytes play a key role in gestational immune tolerance.

B cells. The generation of high-affinity, class-switched antibodies is essential for effective adaptive immunity. Neonatal B cells have increased miR-181b expression compared to adult cells, which is associated with impaired class-switch recombination of IgG and IgA. A murine model of miR-181b deficiency is associated with improved class-switch recombination, demonstrating the importance of miR-181b in this process.[73]

Both innate and adaptive immune cells demonstrate marked differences in both global and site-specific DNA methylation and histone tail modifications over the course of development from preterm neonate to adult (Fig. 3). This is accompanied by differences in microRNA expression based on the stage of development. Each of these epigenetic mechanisms contribute to developmental stage-specific differences in immune cell function and a heightened risk of infection during the neonatal and infant periods.

PRENATAL EXPOSURES

Prenatal exposures can result in long-term alterations in the epigenetic profiles of offspring. This is well demonstrated in the case of in utero famine exposure, where whole blood DNA methylation patterns in adults differ based on whether or not their mother experienced famine during the pregnancy.[74,75] In this section, we will focus on the impact of various prenatal exposures on immune cell epigenetic changes in the offspring.

Toxins and pollutants

Tobacco. Maternal smoking during pregnancy is associated with low birth weight infants, childhood adiposity, neuropsychiatric disorders, and persistent wheezing and asthma in offspring.[22,76–78] Numerous large clinical cohort studies demonstrate that smoking during pregnancy results in differential DNA methylation in neonatal umbilical cord blood.[22,79–83] Maternal smoking is associated with hypomethylation of the *AHRR*, *GFI1*, and *CNTNAP2* loci and hypermethylation of the *MYO1G* and *CYP1A1* loci in neonatal umbilical cord blood, and these findings have been reproduced in multiple studies.[22,80–82] *AHRR* and *CYP1A1* are part of the aryl-hydrocarbon receptor pathway and regulate the response to cigarette hydrocarbons.[84] *MYO1G* and *GFI1* are involved in hematopoiesis, while *CNTNAP2* is involved in nervous system development.[85–87] All of these pathways likely contribute to the negative health consequences related to maternal smoking, and mediation analysis shows that methylation changes at these sites mediates the association between maternal smoking and low birth weight.[22,80] In addition, these differentially methylated sites persist through childhood and adolescence.[79,81,82] Maternal smoking is also associated with differential methylation of the *TSLP* locus in neonatal mononuclear cells, which is associated with the development of childhood atopic dermatitis.[83] Paternal smoking has also been associated with offspring epigenetic changes. Paternal smoking results in altered neonatal DNA

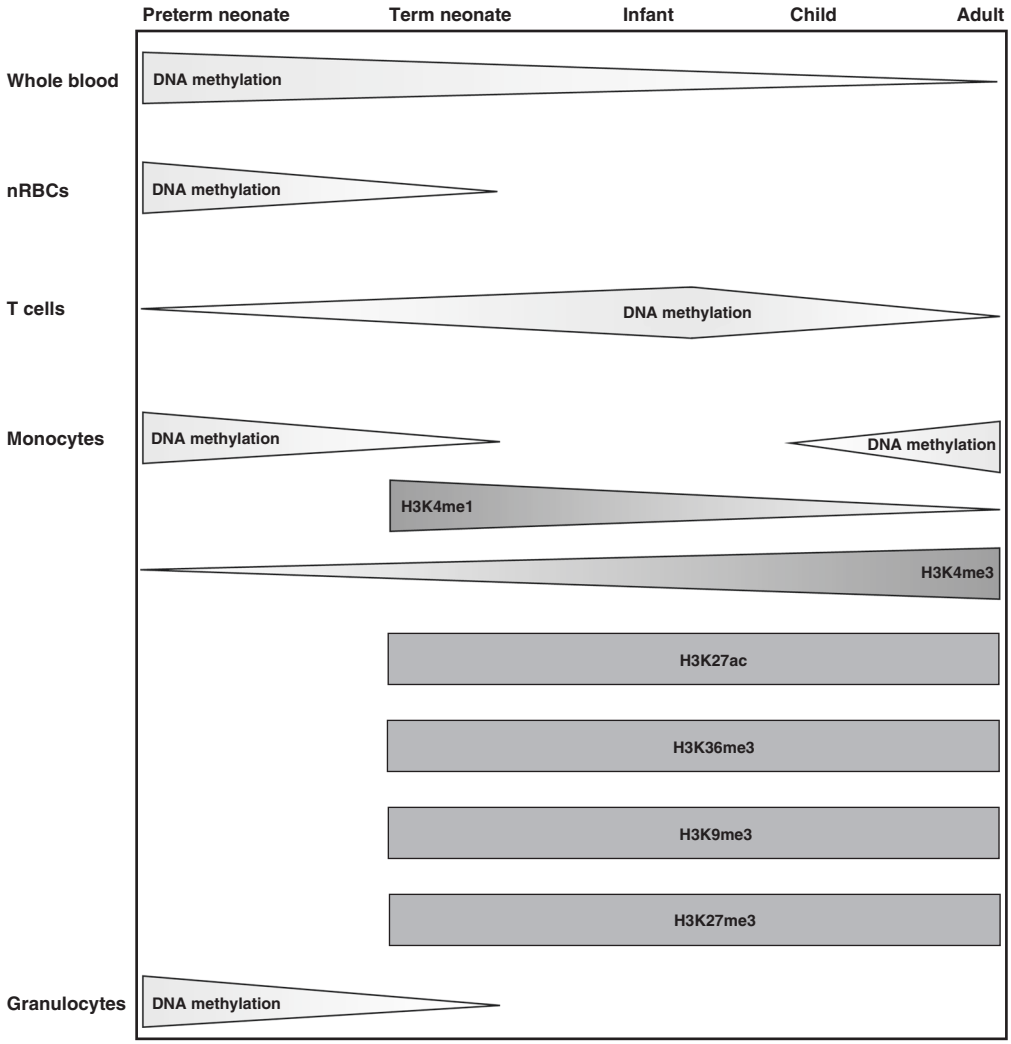

Fig. 3 Summary of global DNA methylation and histone tail modification changes in immune cells over the course of development from preterm neonate to adult. nRBCs nucleated red blood cells.

methylation, with increased methylation of the *LMO2* and *IL10* loci in umbilical cord blood.[88] These methylation changes persist until age 6 and correlate with increased childhood asthma risk.[88] Tobacco use during pregnancy results in increased miR-223 expression in umbilical cord blood, which has implications for offspring myeloid cell development and function.[89] There is strong evidence that tobacco exposure during pregnancy has significant and long-lasting effects on the epigenetic profile of neonatal immune cells, and it is likely this contributes to poor offspring health.

Heavy metals. Mercury and arsenic are known developmental toxicants, and in utero exposure is associated with poor cognitive development in offspring.[90–92] Elevated maternal levels of mercury and arsenic are associated with differential DNA methylation in umbilical cord blood.[93,94] Differentially methylated sites map to pathways involved in antigen processing and presentation, TGF-β signaling, leukocyte migration, and natural killer cell cytotoxicity.[94] In utero arsenic exposure is also associated with increased expression of several immunomodulatory microRNAs, including let-7a, miR-126, miR-16, miR-17, miR-20a, miR-20b, miR-96, and miR-98, in umbilical cord blood of offspring.[95]

Organic compounds. Per- and polyfluoroalkyl substances are man-made endocrine-disrupting compounds commonly used in manufacturing. In utero exposure to these compounds is associated with altered vaccine responses, altered lipid profiles, and increased adiposity in offspring.[96,97] Elevated maternal serum per- and polyfluoroalkyl substance concentrations during pregnancy are associated with differential DNA methylation in offspring mononuclear cells.[98] Genes demonstrating differential DNA methylation are important for growth (*RPTOR*), lipid homeostasis (*PON1*, *PON3*, *CIDEB*, *NR1H2),* and immune function (*RASL11B, RNF39*).[98] Polybrominated diphenyl ether (PBDE) is an organic compound with endocrine-disrupting properties that is found in flame retardants and is known to leach into the environment.[99,100] Maternal exposure to PBDE during pregnancy is associated with cognitive delay in offspring.[100] Elevated maternal levels of PBDE during pregnancy is associated with decreased methylation of the *TNF* locus and increased TNF-α levels in offspring umbilical cord blood.[101]

Air pollution. Air pollution is associated with an increased risk of developing asthma.[102] Nitrogen dioxide is a surrogate marker for air pollution. A meta-analysis of several exposure cohorts found that nitrogen dioxide exposure during pregnancy is associated with differential methylation of the antioxidant genes *CAT* and *TPO* in whole umbilical cord blood.[103] Maternal exposure to the traffic-derived air pollutant polycyclic aromatic hydrocarbon during pregnancy is associated with increased methylation of

the *IFNG* and *ACSL3* loci in offspring mononuclear cells and increased asthma symptoms prior to age 5.[104,105]

These studies show that in utero exposure to toxins and pollutants remodels fetal immune cell epigenetic profiles, and that this remodeling is associated with poor offspring immune health.

Maternal nutrition

Vitamin D. It has recently been recognized that vitamin D impacts DNA methylation.[106] A rat model of gestational vitamin D deficiency demonstrates increased serum DNA methyltransferase activity, increased methylation of the *IFNG* locus, and decreased IFN-γ expression in whole blood of offspring born to vitamin D-deficient mothers.[107] In humans, mononuclear cells from 4- to 6-week-old breastfed infants show differential DNA methylation based on whether their mothers were receiving extra vitamin D_3 supplementation (3800 IU daily starting in late second trimester) or standard of care (400 IU daily).[108] These differentially methylated genes were primarily involved in collagen metabolism and cellular apoptosis.[108]

Folate. Folate acts as a methyl donor in one-carbon metabolism, and sufficient folate levels are necessary for DNA methylation to occur.[109] A mouse model of folate supplementation during pregnancy shows decreased methylation of the *PPARA* locus in offspring colonic tissue compared to offspring of unsupplemented mothers.[110] This is associated with increased susceptibility to experimentally induced colitis in folate-supplemented offspring.[110] Human neonatal CD4+ T cells and myeloid cells demonstrate differential DNA methylation based on maternal folate levels during the third trimester as well.[111] Maternal folate levels are also associated with changes in offspring histone tail modifications. Neonatal CD4+ T cells born to mothers with high gestational folate levels show increased H3 and H4 acetylation at the *GATA3* and *IL9* promoters (associated with Th2 phenotype) compared to neonates born to mothers with low folate levels.[112] This suggests that high maternal folate levels increase chromatin accessibility at key Th2 loci in offspring, which has major implications for subsequent immune and allergic responses.[112]

Fatty acids. Adequate intake of omega-3 polyunsaturated fatty acids is critical for adult immunity. Offspring born to mothers with high fatty fish intake during pregnancy (rich in omega-3 polyunsaturated fatty acids) have a decreased risk of developing allergic diseases during childhood.[113,114] Omega-3 polyunsaturated fatty acids have been shown to influence DNA methylation, which may explain this association.[115] Maternal intake of omega-3 polyunsaturated fatty acids during pregnancy is associated with differential DNA methylation in immune-related pathways in neonatal umbilical cord blood.[116–118] There are no differences in neonatal CD4+ T cell DNA methylation based on gestational omega-3 polyunsaturated fatty acid intake, which suggests that the differences observed in other studies involve other immune subpopulations.[119] Gestational omega-3 polyunsaturated fatty acid supplementation also influences offspring histone tail modifications. CD4+ T cells from neonates born to mothers supplemented with fish oil during pregnancy have increased histone H3 acetylation at the *PRKCZ* promoter (the gene encoding PKCζ, a T cell protein kinase C), decreased histone H3 acetylation at the *TBX21* promoter (Th1 transcription factor) and decreased histone H3/H4 acetylation at the *IL13* promoter (Th2 cytokine) compared to unsupplemented mothers.[120] These findings are associated with a more Th1 phenotype, and could be a plausible explanation for differences in offspring allergy risk.[120]

Maternal health and lifestyle

Maternal obesity and gestational diabetes. Maternal obesity has long-term health consequences for offspring, including an increased risk of obesity, metabolic syndrome, and asthma.[121] Many of these risks are thought to be immune-mediated, and mounting evidence suggests that epigenetics may be involved. Most studies show that maternal pre-pregnancy obesity (defined as BMI > 30) is associated with differential umbilical cord blood immune cell DNA methylation compared to offspring from mothers with a normal pre-pregnancy weight.[40,122–124] This differential methylation persists at least until age 3.[40] Interestingly, only accelerated gestational weight gain during the first 18 weeks of pregnancy is associated with differences in offspring DNA methylation.[15,122,125] This suggests that maternal fat content and deposition are the main driver of these DNA methylation changes.[126] Monocytes from neonates born to obese mothers demonstrate differential DNA methylation compared to neonates born to lean mothers.[127–129] The differential DNA methylation is seen in immune pathways, including myeloid cell migration and adhesion, defense response, and the ability of innate immune cells to activate T cells.[127–129] This is associated with differences in inflammatory gene expression, including decreased *IL1B* expression in monocytes from neonates of obese mothers.[127,128] These findings suggest that DNA methylation contributes to maternal obesity-related neonatal monocyte hypo-responsiveness.[127] Gestational diabetes also influences umbilical cord blood DNA methylation.[130] Offspring from gestational diabetics have hypermethylation of genes involved in antigen processing and presentation with hypomethylation of genes involved in development.[130] This is likely to influence offspring immune responses and metabolic reprogramming. Maternal obesity-related changes in offspring epigenetic profiles may or may not involve microRNA expression. One study shows decreased serum miR-155, miR-181a, and miR-221 levels in neonates born to obese mothers[131] while another finds no difference in serum microRNA levels between neonates born to obese or lean mothers.[132] A gestational low glycemic index dietary intervention altered neonatal umbilical cord blood DNA methylation, with a large impact on DNA methylation in immune-related genes.[133] Similarly, mononuclear DNA methylation patterns differed between siblings born before and after maternal bariatric surgery.[134] These DNA methylation differences included multiple immune pathways, and were associated with lower BMI, fasting insulin levels, blood pressure, and CRP in children born following the bariatric surgery.[134] These results are encouraging, and suggest that active treatment or resolution of maternal obesity prior to or during pregnancy can alter offspring epigenetics and subsequent health outcomes.

Maternal type 1 diabetes. Offspring born to mothers with type 1 diabetes are protected against the development of autoantibodies against (pro)insulin, and this is associated with a lower risk of developing type 1 diabetes during childhood.[135] As an explanation of these findings, neonates born to mothers with Type 1 diabetes have hypomethylation of the *INS* (insulin) gene with reduced CD4+ T cell responses to insulin compared to neonates born to nondiabetic mothers.[135]

Gestational hypertension. Neonates born to mothers with gestational hypertension demonstrate early life endothelial dysfunction and have an increased risk of hypertension in adulthood.[136,137] Neonates born to hypertensive mothers have increased miR-146a expression in umbilical vein endothelial cells compared to neonates with normotensive mothers.[138] Elevated miR-146a expression reduced in vitro vascular tube formation, but miR-146a inhibition was able to rescue appropriate tube formation.[138] This suggests that miR-146a links maternal hypertension to offspring vascular development and function.

Psychiatric and socioeconomic factors. CD3+ T cells from neonates born to mothers with symptomatic depression during pregnancy have differential DNA methylation compared to neonates born to mothers without depression.[139] These

differentially methylated sites cluster in immune pathways, including leukocyte activation, migration and differentiation, and T cell signaling. Several of these differentially methylated sites are present in the hippocampus of adults born to mothers with depression, suggesting that maternal depression results in lifelong epigenetic alterations in offspring.[139] Prenatal stress, defined as maternal bereavement, natural disaster, or traumatic experience, is associated with increased BMI and risk of overweight/obesity in offspring.[140–142] Prenatal stress is associated with increased methylation of the *IL6* locus in umbilical cord blood, and this is associated with increased offspring adiposity at age 4–6.[143] Women who experienced childhood maltreatment demonstrate differences in mononuclear cell DNA methylation at selected stress-response-associated genes.[144] Mononuclear cells from neonates born to mothers with childhood maltreatment showed no difference in DNA methylation at any of these sites, suggesting that these epigenetic patterns are not transmitted to the next generation.[144]

Farming exposure. Maternal exposure to farming decreases the risk of allergic disease in offspring.[145,146] Neonates born to mothers with farm milk exposure have hypomethylation of the *FOXP3* promoter in mononuclear cells.[147] This is associated with an increased number of neonatal Tregs and improved Treg function, which is thought to contribute to this decreased allergy risk.[147]

Infection and inflammation

Maternal inflammation and chorioamnionitis. Chorioamnionitis is infection and/or inflammation of the chorion, amnion, and placenta. Chorioamnionitis is associated with altered neonatal immune responses and the development of persistent wheezing and asthma during childhood.[148–150] This suggests that early life inflammatory exposures have pervasive effects on the developing immune system and there is evidence that epigenetics plays a role in this process. Higher levels of circulating maternal cytokines during the first trimester are associated with decreased methylation of the *MEG3* locus in neonatal mononuclear cells.[151] *MEG3* is a long non-coding RNA that mediates the transition from epithelial to mesenchymal cells and acts as a tumor suppressor, and it is plausible it could contribute to maternal inflammation-induced lung dysfunction.[152] Mononuclear cells from chorioamnionitis-exposed neonates demonstrate differential DNA methylation at multiple genes involved in asthma development, immune regulation, and inflammation.[153] Fetuses exposed to acute chorioamnionitis demonstrate increased miR-223 in the thymus, lung, and liver compared to unexposed fetuses.[154] miR-223 has immunomodulatory effects, and is known to regulate myeloid cell proliferation and differentiation.[155] Chorioamnionitis exposure has also been shown to cause a global gain in the activating histone tail modification H3K4me3 in neonatal monocytes.[148] This gain is primarily in introns and intergenic regions rather than promoters, and chorioamnionitis-exposed monocytes actually experience a loss of promoter-site H3K4me3. These changes are associated with alterations in gene transcription and decreased pro-inflammatory cytokine expression in chorioamnionitis-exposed monocytes, including IL-1β, IL-6, and IL-8.[148] These studies provide compelling evidence that epigenetic mechanisms contribute to chorioamnionitis-induced neonatal immune dysfunction.

Congenital infection. Perinatally acquired human immunodeficiency virus (HIV) has persistent effects on long-term health outcomes, including cognitive deficits, metabolic abnormalities, and renal complications, even when antiretroviral therapy is started early.[156–158] Peripheral blood from 4- to 9-year-old children with perinatally acquired HIV demonstrate differential DNA methylation compared to uninfected controls.[159] Differentially methylated genes are in pathways important for adaptive immunity, and these differences may contribute to some of the long-term health effects experienced by children with perinatally acquired HIV.[159] Congenital Zika virus infection is associated with severe microcephaly and poor neurocognitive outcomes.[160] Toddlers with congenital Zika virus infection and microcephaly have differential whole blood DNA methylation compared to unexposed normocephalic children.[161] This includes hypomethylation of *RABGAP1L*, *MX1* and *ISG15*.[161] *RABGAP1L* is involved in brain development and *MX1* and *ISG15* are involved in viral host immunity and work to inhibit Zika virus replication.[162–164] These studies suggest that congenital infections alter the offspring epigenome, which may contribute to the long-term health consequences of perinatally acquired infections.

Gestational probiotics. Supplementation with the probiotic *Lactobacillus reuteri* decreases allergen responsiveness during infancy.[165] CD4+ T cells from neonates born to *Lactobacillus reuteri* supplemented mothers demonstrate global DNA hypomethylation compared to neonates born to unsupplemented mothers.[60] These hypomethylated areas are enriched in immune-related pathways, including chemotaxis, PI3K-Akt, MAPK, and TGF-β signaling, which likely influences later allergy development.[60]

Glucocorticoid exposure. Prenatal dexamethasone treatment is used to reduce virilization in female fetuses with suspected or confirmed congenital adrenal hyperplasia and prenatal administration of betamethasone is the standard of care for women at risk for preterm delivery.[166] Prenatal glucocorticoid exposure also poses potential risks to the offspring, with prenatal dexamethasone exposure being associated with an altered immune phenotype during adolescence.[167] CD4+ T cells from adolescents with first-trimester dexamethasone exposure demonstrate differential DNA methylation compared to unexposed adolescents.[166] Differentially methylated genes are involved in immune pathways, including IL-1 production and secretion, T cell receptor complex, macrophage activation, and granulocyte activation.[166] Complementary studies in rats show that in utero dexamethasone exposure alters histone tail modifications in the spleens of adult offspring. There is a decrease in the activating modifications H3K9ac and H3K36me3 at the *IFNG* locus and a decrease in the activating modifications H3 lysine acetylation, H3K9/14ac, H3K4me1, H3K4me3, and H3K36me3 at the *TNF* locus in adult offspring with in utero dexamethasone exposure.[168,169] These findings are associated with impaired IFN-γ and TNF-α expression, suggesting that prenatal dexamethasone exposure has a long-lasting impact on offspring immune function by altering immune cell epigenetic profiles.[168,169]

It is clear that prenatal exposures alter offspring epigenetic profiles and influence subsequent immune responses. The impact of prenatal exposures on offspring epigenetics is summarized in Fig. 4.

EARLY LIFE EXPOSURES

Early life exposures have a major impact on the long-term health of an individual. Early life exposures are linked to adult asthma, cardiovascular disease, metabolic syndrome, and cancer risk.[170–172] In this section, we will discuss the impact of early life exposures, including nutrition, infection, environment, and socioeconomic factors, on immune epigenetic reprogramming.

Mode of delivery

Mode of delivery (vaginal or cesarean section) does not have a convincing impact on neonatal immune cell DNA methylation.[173–175] The only study that demonstrates DNA methylation changes based on mode of delivery also shows that these methylation changes resolve by 5 days of age.[174] This rapid resolution calls into question the biological significance of these changes.

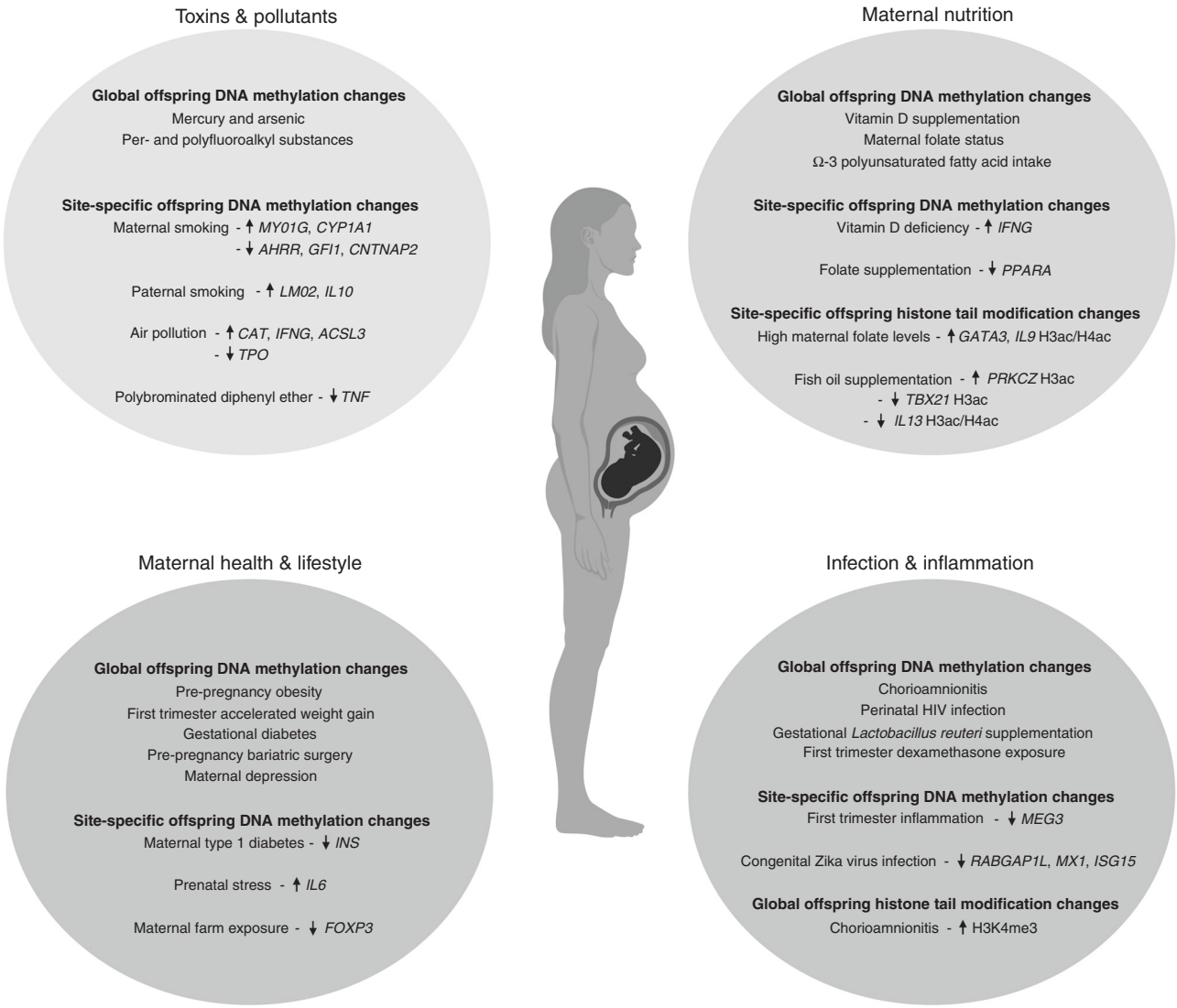

Fig. 4 Schematic representation of offspring DNA methylation and histone tail modification changes following prenatal exposures. Created with BioRender.com.

Nutrition

Breastfeeding. Breastfeeding has numerous well known benefits to the offspring, including improved neurodevelopmental outcomes and a decreased risk of childhood allergic diseases, including asthma.[176,177] Breastfeeding for greater than 6 months is associated with differences in peripheral blood DNA methylation at 10 years of age, including hypermethylation of *SNX25*.[178] SNX25 regulates TGF-β signaling, which is involved in allergy development. These methylation differences are not present at birth and do not persist at 18 or 26 years of age.[178] This suggests that breastfeeding drives these postnatal DNA methylation changes during a time period crucial for allergy development. The effect of breastfeeding on offspring microRNA expression and histone tail modifications has not been studied, but the role of breastmilk microRNAs in neonatal and infant immune system development has recently been comprehensively reviewed.[179,180]

Fatty acids. As previously described, omega-3 polyunsaturated fatty acids influence DNA methylation and gestational intake is associated with altered offspring DNA methylation.[115–118] However, supplementing infants with omega-3 polyunsaturated fatty acids in the form of fish oil for 9 months is not associated with differences in mononuclear cell DNA methylation.[181] This suggests that gestation is a critical time window in which fatty acids can reprogram offspring epigenetics, but that this window closes following birth.

Vitamin D. Elevated umbilical cord blood vitamin D levels are associated with a decrease in the repressive histone tail modifications H3K9me3 and H3K27me3 at the *TSLP* promoter and adjacent enhancer regions.[182] This is associated with enhanced *TSLP* expression and an increased incidence of wheezing in the first 3 years of life compared to neonates with low vitamin D levels at birth.[182] Vitamin D not only influences epigenetics during gestation and early life but also during adolescence.[108,183] Adolescents with severe vitamin D deficiency demonstrate differential mononuclear cell DNA methylation compared to vitamin D sufficient adolescents.[183]

Malnutrition. Undernutrition affects nearly 25% of children worldwide, and is associated with vaccine failure and cognitive impairment.[184] Children with undernutrition at 1 year of age have global remodeling of the activating histone modification H3K4me3 in mononuclear cells compared to well-nourished children.[185] This remodeling is associated with decreased promoter-site H3K4me3 with global redistribution to other

Table 1. MicroRNA expression in neonatal sepsis.

MicroRNA	Tissue	Increased in	Compared to	Known function	Ref.
miR-101	Mononuclear cells	Controls	Gram-negative sepsis	Cancer pathogenesis	192
miR-106a	Mononuclear cells	Controls	Gram-negative sepsis	Monocyte proliferation and differentiation	192
miR-1184	Mononuclear cells	Gram-negative sepsis	Controls	Cancer pathogenesis	192
miR-122	Mononuclear cells	Gram-positive sepsis	Controls	Liver homeostasis and hepatocyte innate immunity	192
miR-126	Mononuclear cells	Controls	Gram-negative sepsis	Pathogen-associated innate immune responses and Th2 differentiation	192
miR-1299	Mononuclear cells	Gram-positive sepsis	Controls	Th17 differentiation[365]	192
miR-132	Serum	Controls	Clinical early onset sepsis	B cell differentiation and T cell signaling	191
miR-141	Mononuclear cells	Controls	Gram-negative sepsis	Tumor suppressor	192
miR-142	Mononuclear cells	Controls	Gram-negative sepsis	B cell activation	192
miR-143	Mononuclear cells	Controls	Gram-negative sepsis	Tumor suppressor	192
miR-146b	Mononuclear cells	Controls	Gram-negative sepsis	B and T cell responses	192
miR-15a	Mononuclear cells	Controls	Gram-negative sepsis	Macrophage differentiation and suppression of LPS-induced inflammation	192
	Serum	Culture-positive late onset sepsis	Controls		193
miR-16	Mononuclear cells	Controls	Gram-negative sepsis	Macrophage differentiation and suppression of LPS-induced inflammation	192
	Serum	Culture-positive late onset sepsis	Controls		193
miR-17	Mononuclear cells	Controls	Gram-negative sepsis	Myeloid cell proliferation and differentiation and B cell differentiation	192
miR-181a	Mononuclear cells	Controls	Gram-negative sepsis	B and T cell differentiation and T cell responses	192
miR-182	Mononuclear cells	Controls	Gram-positive sepsis	T cell clonal expansion and granulocyte differentiation	192
miR-185	Mononuclear cells	Gram-positive sepsis	Controls	Inhibits angiogenesis	192
miR-19a	Mononuclear cells	Controls	Gram-negative sepsis	T cell function	192
miR-20a	Mononuclear cells	Controls	Gram-negative sepsis	Monocyte proliferation and differentiation	192
miR-20b	Mononuclear cells	Controls	Gram-negative sepsis	Th17 differentiation	192
miR-210	Mononuclear cells	Controls	Gram-positive sepsis	B cell activation	192
miR-22	Mononuclear cells	Controls	Gram-negative sepsis	T cell responses	192
miR-222	Mononuclear cells	Controls	Gram-positive sepsis	Cytokine responses	192
miR-223	Serum	Controls	Clinical early onset sepsis	Myeloid cell proliferation and differentiation	191
miR-26a	Serum	Controls	Culture-positive sepsis	Inhibition of IL-6 expression	190
	Mononuclear cells	Controls	Gram-negative sepsis		192
miR-29a	Mononuclear cells	Controls	Gram-negative sepsis	Common myeloid progenitor differentiation and response to bacteria	192
miR-30a	Mononuclear cells	Controls	Gram-negative sepsis	Th17 differentiation	192
miR-33a	Mononuclear cells	Controls	Gram-negative sepsis	Antiviral immunity	192
miR-96	Mononuclear cells	Controls	Gram-positive sepsis	Tumor suppressor	192

genomic sites. Pathways containing remodeled H3K4me3 include cytokine signaling and adaptive immunity, which may contribute to insufficient vaccine responses in undernourished children.[185]

Infection and inflammation

Sepsis. Preterm neonates diagnosed with clinical sepsis have differential mononuclear cell DNA methylation compared to healthy preterm neonates.[186] Hypomethylated genes are enriched in pathways involved in neutrophil activation and degranulation, leukocyte migration, and cytokine production. Conversely, hypermethylated genes are enriched in pathways involved in T cell activation and differentiation, T cell receptor signaling, and cytokine production. TREM1 has been proposed as an early biomarker of neonatal sepsis, and hypomethylation of the *TREM1* locus is noted in septic preterm neonates.[186–188] S100A8 is an alarmin known to prevent expansion of inflammatory monocyte populations in neonatal sepsis, and hypomethylation of the *S100A8* locus is detected in septic preterm neonates.[186,189] Differential microRNA expression has also been described in neonatal sepsis, and appears to differ based on the organism causing sepsis.[190–193] Multiple studies demonstrate decreased miR-26a expression in septic neonates.[190,192] IL-6, which is a validated biomarker for the early diagnosis of neonatal sepsis, is a direct target of miR-26a and sepsis-induced downregulation of miR-26a may contribute to elevated IL-6 levels.[190,194] A detailed list of microRNA expression in neonatal sepsis can be found in Table 1. These studies suggest that sepsis-induced changes in DNA methylation and microRNA expression contribute to phenotypes described in neonatal sepsis, and are attractive therapeutic targets.

Viral respiratory infections. Early life viral respiratory infections are associated with long-term health consequences, including persistent wheezing and asthma.[195–197] Children who develop two or more lower respiratory tract infections within the first year of life have increased methylation of the *PRF1* locus (involved in immunity and cytolysis) in umbilical cord blood mononuclear cells compared to children with no infections.[198] This suggests that susceptibility to early life lower respiratory tract viral infections may be influenced by DNA methylation changes at birth. Interestingly, 3–4 year old children who were hospitalized for severe respiratory syncytial virus (RSV) infection prior to age 2 demonstrate hypomethylation of the *PRF1* loci in whole blood.[199] It is unclear what the methylation status of the *PRF1* locus was in these children at birth, but it is plausible that the methylation status of *PRF1* was altered during the severe RSV infection as an explanation for the difference in these findings. Rhinovirus also results in differential DNA methylation in children with asthma, which is thought to link this early life respiratory infection to asthma development and exacerbation.[200,201] Acute RSV infection is also associated with alterations in immunomodulatory microRNA expression.[202–208] These findings are highlighted in Table 2. Multiple studies demonstrate upregulation of miR-155 in nasal mucosa from RSV infected children, and demonstrate that higher miR-155 levels are associated with reduced disease severity.[203,208] miR-155 is known to regulate myeloid cell activation, T cell responses and cytokine signaling.[155,209] None of the additional differentially expressed microRNAs have been demonstrated in more than one study.[202–208] Similar to RSV, children with rhinovirus infection have increased miR-155 in nasal secretions compared to healthy controls.[208,210] However, nasal mucosa demonstrates differential expression of multiple other immunomodulatory microRNAs between children with rhinovirus and RSV infections.[211] This suggests that each of these respiratory viruses have a unique impact on host epigenetics, but that these changes impact similar mechanisms in the development of childhood asthma.

Hepatitis B. Children with the hepatitis B e antigen (HBeAg), which is associated with active infection, have increased plasma miR-28-5p, miR-30a-5p, miR-30e-3p, miR-378a-3p, miR-574-3p, and let-7c and decreased miR-654-3p compared to antigen negative controls. These microRNAs target liver-specific genes, and may contribute to the higher risk of hepatocellular carcinoma and cirrhosis seen in patients with chronic hepatitis B infection.[212] Different plasma microRNA profiles are also observed during different stages of chronic pediatric hepatitis B infection.[213] Immune tolerant children (HBeAg positive, >20,000 IU/mL viral DNA, normal liver function) demonstrate the highest levels of miR-99a-5p, miR-100-5p, miR-122-5p, miR-122-3p, miR-125b-5p, miR-192-5p, miR-192-3p, miR-193b-3p, miR-194-5p, miR-215, miR-365a-3p, miR-455-5p, miR-483-3p and 885-5p. Immune active children (HBeAg positive, >20,000 IU/mL viral DNA, elevated liver function tests) have intermediate levels and immune inactive children (HBeAg negative, <2000 IU/mL viral DNA, normal liver function) have the lowest levels of these microRNAs. This demonstrates that microRNA levels are inversely correlated with immunologic control of chronic pediatric hepatitis B infection.[213]

Tuberculosis. Children with the active contagious form of tuberculosis (TB) have global peripheral blood DNA hypomethylation compared to uninfected controls.[214] This was proposed as a potentially useful biomarker to monitor disease progression and treatment efficacy. Pediatric patients with active TB also demonstrate differential microRNA expression compared to healthy controls.[215,216] There are increased levels of miR-21, miR-29a, miR-31, miR-155, and decreased levels of miR-146a in plasma from pediatric patients with active TB.[216] It is unclear what impact active TB has on miR-31 expression, as one study demonstrates increased miR-31 in patients with active TB[216] while another demonstrates decreased expression.[215] MicroRNA expression has been proposed as a potential diagnostic biomarker for pediatric TB, but further validation of microRNA levels in active TB is required before this can be put into practice.

Parasites. Parasitic infections are common in developing countries and result in altered immunity and poor vaccine responses.[217,218] CD4+ T cells from children with active *Schistosoma haematobium* and/or *Ascaris lumbricoides* infection have differential DNA methylation compared to age-matched uninfected controls.[219] Hypermethylated genes included numerous transcription factors and other immunologically important genes, including *IFNGR1*, *TNFS11*, *RELT*, *IL12RB2*, and *IL12B*. These findings are associated with downregulation of IFN-γ inducible genes in infected individuals, which may explain the poor vaccine responses seen in helminth-infected children. These findings persist for at least 6 months after deworming is complete, which could impact future vaccination strategies.[219]

Vaccines. Differences in DNA methylation are associated with the strength of the immune response to the 13-valent pneumococcal conjugate vaccine.[32] Infants who are high responders to the vaccine (based on IgG response) have hypomethylation of the *HLA-DPB1* locus and hypermethylation of the *IL6* locus in peripheral blood compared to low responders.[32] These findings suggest that epigenetics influences vaccine responses, and has the potential to inform vaccine dosing and administration schedules.

Pollutants

Pollution appears to alter the chromatin landscape in both innate and adaptive immune cells. Children exposed to secondhand smoke and ambient air pollution have hypermethylation of the *IFNG* locus in effector T cells and hypermethylation of the *FOXP3* locus in Tregs. This hypermethylation is associated with decreased expression of both of these genes in a cell-specific manner, resulting in a Th2 phenotype.[220] Additionally, children with either high polycyclic aromatic hydrocarbon or ambient air pollution exposure have increased *FOXP3* methylation with associated Treg dysfunction.[221,222] Alveolar macrophages from children with severe asthma and passive smoke exposure have significantly lower expression of the histone deacetylase HDAC2 with an associated decrease in dexamethasone-induced inhibition of inflammation compared to children with severe asthma without passive smoke exposure.[223] These findings are thought to contribute to the adverse health consequences of these environmental exposures, including the development and exacerbation of asthma symptoms.

Socioeconomic factors

Socioeconomic status is one of the strongest predictors of physical and mental health, and is known to influence immune responses.[224,225] Family income, parental education, and family psychosocial adversity are associated with differential DNA methylation in buccal epithelial cells of kindergarten-aged children. Differentially methylated genes are involved in immune processes, including T cell responses and immunoglobulin function.[226] This provides some mechanistic insight into social determinants of health outcomes.

These findings make a strong case that early life exposures have a marked impact on immune epigenetics and subsequent health outcomes. The impact of early life exposures on epigenetic reprogramming is summarized in Fig. 5.

DISEASE STATES

Epigenetics are implicated in a wide variety of disease processes, including cancer, autoimmune disease, neuropsychiatric conditions, and asthma, among many others.[227,228] In this section we

Table 2. MicroRNA expression in childhood viral respiratory infections.

MicroRNA	Tissue	Increased in	Compared to	Known function	Ref.
let-7d	Nasal mucosa	RSV	Controls	Tumor suppressor	203
miR-101	Natural killer cells	RSV	Controls	Cancer pathogenesis	202
miR-103	Natural killer cells	RSV	Controls	Cancer pathogenesis	202
miR-106b	Whole blood	RSV	Controls	Cancer pathogenesis	205
miR-10a	Whole blood	Severe RSV	Mild RSV	Helper T cell function	204
miR-122	Whole blood	Controls	RSV	Liver homeostasis and hepatocyte innate immunity	205
miR-125a	Nasal mucosa	Controls	RSV	Treg-mediated immune homeostasis	203
miR-125b	Nasal mucosa	Controls	RSV	TGF-β signaling, myeloid cell proliferation and differentiation and B cell activation	203
	Whole blood	Severe RSV	Mild RSV		204
miR-1271	Whole blood	Severe RSV	Mild RSV	Tumor suppressor	204
miR-140	Nasal mucosa	Controls	RSV	T cell differentiation	206
	Whole blood				
miR-149	Nasal mucosa	Rhinovirus	RSV	Cancer pathogenesis	211
miR-155	Nasal mucosa	RSV	Controls	NF-κB signaling, myeloid cell activation, T and B cell responses	203
	Nasal mucosa	Rhinovirus or RSV			208
	Nasal mucosa	Rhinovirus			210
miR-16	Nasal mucosa	RSV	Controls	Macrophage differentiation and suppression of LPS-induced inflammation	203
miR-197	Nasal mucosa	Rhinovirus	RSV	Cancer pathogenesis	211
miR-199b	Natural killer cells	Controls	RSV	Tumor suppressor	202
miR-203a	Nasal mucosa	RSV	Controls	Tumor suppressor	203
miR-20b	Whole blood	RSV	Controls	Th17 differentiation	205
miR-221	Natural killer cells	RSV	Controls	Cytokine responses	202
miR-222	Natural killer cells	RSV	Controls	Cytokine responses	202
miR-26b	Mononuclear cells	RSV	Controls	Tumor suppressor	207
miR-27b	Nasal mucosa	Controls	RSV	TGF-β signaling	203
miR-296	Nasal mucosa	Rhinovirus	RSV	Tumor suppressor	211
miR-29c	Nasal mucosa	Controls	RSV	Viral-associated immune responses	203
miR-3074	Natural killer cells	RSV	Controls	Myoblast homeostasis	202
miR-30b	Whole blood	Severe RSV	Mild RSV	Increases IL-10 expression	204
miR-30d	Natural killer cells	RSV	Controls	Tumor suppressor	202
miR-31	Nasal mucosa	RSV	Controls	Treg function	203
miR-320d	Whole blood	Controls	RSV	Inhibition of IL-8 expression	205
miR-320e	Whole blood	Controls	RSV	Tumor suppressor	205
miR-342	Whole blood	RSV	Controls	Antiviral immunity	205
miR-34b	Nasal mucosa	Controls	RSV	Antibacterial immunity	203
miR-34c	Nasal mucosa	Controls	RSV	Antibacterial immunity	203
miR-370	Natural killer cells	RSV	Controls	Viral-associated immune responses	202
miR-379	Natural killer cells	RSV	Controls	Tumor suppressor	202
miR-429	Nasal mucosa	Controls	RSV	Tumor suppressor	203
miR-504	Nasal mucosa	Rhinovirus	RSV	Tumor suppressor	211
miR-873	Natural killer cells	RSV	Controls	NF-κB signaling	202
miR-877	Whole blood	Controls	RSV	Cytokine responses	205
miR-92b	Whole blood	Controls	RSV	Cancer pathogenesis	205

RSV-respiratory syncytial virus.

will review the contribution of epigenetics to pediatric diseases with a known immune component.

Genetic syndromes

Missense variants in the DNA methyltransferase gene *DNMT3B* results in immunodeficiency, centromeric instability, facial anomalies syndrome (ICF1). Patients with ICF1 have hypomethylation of pericentric regions of chromosomes 1, 9, and 16 in mitogen-stimulated lymphocytes, which is associated with hypogammaglobulinemia, intrinsic T cell defects, and a heightened risk of opportunistic infections.[229,230] Missense or nonsense variants in the *TET2* gene, which promotes DNA methylation, results in whole peripheral blood DNA hypermethylation. This is associated with abnormal T and B cell function, childhood immunodeficiency, and

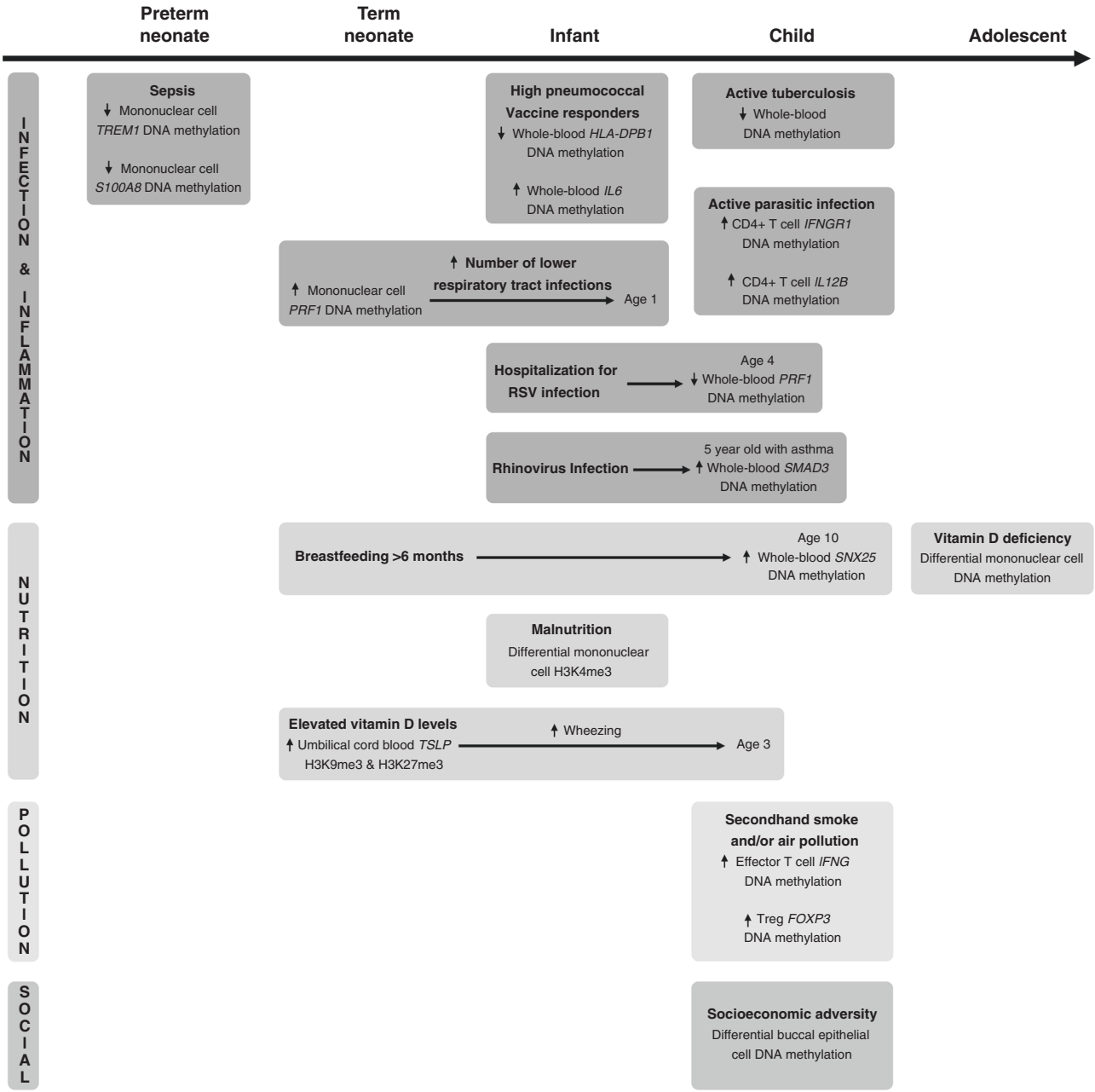

Fig. 5 Summary of the impact of early life exposures on DNA methylation and histone tail modifications throughout childhood. Created with BioRender.com. RSV respiratory syncytial virus, Treg regulatory T cell.

lymphoma development.[231] Kabuki syndrome is a rare disease caused by pathogenic variants in either the H3K4 methyltransferase *KMT2D* (MLL2) or the lysine-specific demethylase *KDM6A*. Kabuki syndrome is characterized by distinctive facial features, intellectual disability, short stature, skeletal anomalies, and the persistence of fetal fingertip pads. Kabuki syndrome is associated with recurrent ear, nose, and throat infections, abnormal immunoglobulin secretion, and poor vaccine responses.[232,233]

Atopic diseases

Th2 immune responses, characterized by IL-4, IL-5, IL-9, and IL-13 expression, play a crucial role in the pathogenesis of asthma and atopy.[234] Allergen exposure also stimulates Th2 cytokine expression, which amplifies Th2 responses in atopic individuals and leads to disease exacerbations.[235] Th1 and Treg responses are downregulated in asthma and other atopic diseases.[236] Many studies have evaluated epigenetic mechanisms in asthma and atopy with inconsistent results.[228,237,238] Here we will focus on the role of epigenetics in pediatric asthma and other atopic diseases.

General atopy. IgE is a central mediator of atopic (allergic) inflammation. High IgE levels are associated with hypomethylation of numerous gene loci, including the Th2-associated loci *IL5RA* and *IL4*, in immune cells of atopic children and young adults.[239,240] DNA methylation also serves as a molecular marker for biologic aging, and DNA methylation age acceleration during early childhood is associated with higher serum total IgE and an increased risk of atopic sensitization.[241,242]

Asthma. Umbilical cord blood demonstrates differential DNA methylation between children who do and do not develop asthma during childhood.[243–247] This includes hypermethylation

Table 3. MicroRNA expression in pediatric atopic asthma.

MicroRNA	Tissue	Increased in	Compared to	Known function	Ref.
let-7e	Whole blood	Severe asthma	Controls	Vascular endothelial cell inflammatory responses	269
miR-106b	Plasma	Asthma	Controls	Cancer pathogenesis	262
miR-126	Plasma	Asthma	Controls	Pathogen-associated innate immune responses and Th2 differentiation	263
miR-146a	Plasma	Asthma	Controls	Myeloid cell, B cell and T cell responses	262,264
miR-148b	Nasal mucosa	Asthma	Controls	Dendritic cell responses	256
miR-21	Plasma	Asthma	Controls	Myeloid cell proliferation and differentiation, B cell activation, Th17 differentiation, suppresses IL-12p35 expression	264,266
miR-22	Whole blood	Controls	Dust mite-induced asthma	T cell responses	268
miR-221	Lymphocytes	Asthma	Controls	Cytokine responses	265
	Whole blood				267
miR-485	Lymphocytes	Asthma	Controls	Antiviral immunity	265
miR-497	Whole blood	Severe asthma	Controls	Tumor suppressor	269
miR-513a	Whole blood	Controls	Dust mite-induced asthma	Tumor suppressor	268
miR-604	Whole blood	Severe asthma	Mild asthma	Chronic hepatitis B infection	269
miR-625	Whole blood	Controls	Dust mite-induced asthma	NF-κB signaling	268
miR-638	Whole blood	Severe asthma	Mild asthma	Tumor suppressor	269
miR-98	Whole blood	Severe asthma	Controls	T cell differentiation	269

of the known asthma-associated genes *SMAD3* and *ORDML3* and the cytokine *IL2* in children who subsequently develop asthma.[244,246,247] These findings suggest that DNA methylation patterns at birth contribute to asthma susceptibility during childhood. Differential DNA methylation patterns are also noted in immune cells after the development of asthma.[245,248–251] This includes hypomethylation of the asthma-associated gene *ORDML3* and the Th2-associated genes *IL13* and *IL5RA*, with hypermethylation of the Treg-associated gene *FOXP3* and the Th1-associated gene *IFNG*.[245,248,250,251] Respiratory and buccal epithelial cells from children with asthma also demonstrate differential methylation at genes with a known role in epithelial barrier function or asthma pathogenesis compared to non-asthmatic children.[245,249,252–258] This includes hypermethylation of the *IFNG* locus in asthmatic children.[258] Allergen-specific immunotherapy is a highly effective treatment for children with allergic asthma.[259] Dust mite allergen-specific immunotherapy increases methylation of the *IL4* locus in mononuclear cells from children with asthma, which is associated with decreased IL-4 expression and decreased sensitivity to dust mite allergen.[260] Taken together, these findings demonstrate that DNA methylation plays a critical role in the pathogenesis of childhood asthma and that targeting immune cell DNA methylation leads to an improvement in symptoms.

MicroRNA expression and histone tail modifications may also contribute to the pathogenesis of childhood asthma. An association study found that polymorphisms of the *miR-146a* locus are associated with the development of asthma.[261] Numerous studies also demonstrate differential microRNA expression between asthmatic children and non-asthmatic controls.[256,262–269] These differences are outlined in Table 3. Elevated levels of the immunomodulatory microRNAs miR-146a, miR-21, and miR-221 have been found in the peripheral blood of asthmatic children in multiple studies.[262,264–267] CD4+ T cells from children with asthma have increased H3 and H4 acetylation at the Th2 locus *IL13* and increased H3 acetylation at the Treg locus *FOXP3* compared to healthy controls.[270] Alveolar epithelial cells from children and young adults with asthma have an increase in the activating histone tail modification H3K18ac at the promoter sites of *TP63*, *EGRF1* and *STAT6*.[271] These genes are important for epithelial repair and tissue maintenance, and increased H3K18ac near their promoters may explain the elevated levels of these genes found in asthmatic airway epithelium.[272–276] These findings implicate epigenetics in the development of asthma, and suggest that several microRNAs may be useful biomarkers of disease.

Allergic rhinitis. Little is known about epigenetics in allergic rhinitis, but two studies show that respiratory epithelial cells from children with allergic rhinitis have differential DNA methylation compared to non-allergic children.[277,278] These differentially methylated sites are enriched in pathways involved in IL-2 signaling, T cell receptor signaling, and bacterial invasion of epithelial cells.[277,278]

Atopic dermatitis. Children with atopic dermatitis do not have global DNA methylation differences in whole blood, T cells, or B cells compared to healthy controls.[279] However, increased expression of the atopic dermatitis associated gene *FCER1G* in children and young adults with atopic dermatitis is associated with hypomethylation of the *FCER1G* promoter in monocytes.[280,281] Additionally, epidermal lesions from pediatric patients with atopic dermatitis demonstrate differential DNA methylation compared to non-atopic children.[279,282] This includes hypomethylation of the atopy associated gene *TSLP* in children with atopic dermatitis.[282] Differential microRNA expression has also been shown in children with atopic dermatitis.[283,284] Elevated serum levels of miR-203 and miR-483-5p, decreased urine miR-203, and elevated miR-155 in skin lesions are found in children with active disease.[283,284] These findings indicate that epigenetics may contribute to the pathogenesis of atopic dermatitis, but more research is needed.

Eosinophilic esophagitis. Almost nothing is known about epigenetic changes in eosinophilic esophagitis. There is a single study showing increased miR-21 in esophageal tissue and serum from pediatric patients with eosinophilic esophagitis compared to healthy controls.[266]

Food allergy. Similar to asthma, umbilical cord blood demonstrates differential DNA methylation between children who do and do not develop food allergy during childhood.[285,286] Many of these differentially methylated sites remain at 12 months of age, suggesting that this predisposing epigenetic landscape remains stable during early life.[286] Children with IgE-mediated food allergy, including cow's milk allergy and peanut allergy, demonstrate differential immune cell DNA methylation compared to non-allergic children.[287–291] Food allergic children demonstrate hypomethylation of the Th2-associated genes *IL5RA* and *IL4* and hypermethylation of the Th1 associated gene *IFNG* and the Treg associated gene *FOXP3*.[288–290,292] DNA methylation patterns also vary by reaction severity amongst patients with peanut allergy.[293] These DNA methylation differences have been used to develop a prediction tool for childhood food allergy, which outperforms traditional allergen-specific IgE and skin prick testing.[294] Effective treatments for childhood food allergy have also been shown to impact epigenetics. Young children with IgE-mediated cow's milk allergy who receive 12 months of an extensively hydrolyzed casein formula containing the probiotic *Lactobacillus rhamnosus* GG have hypomethylation of the *FOXP3* and *IFNG* loci and hypermethylation of the *IL4* and *IL5* loci in CD4+ T cells compared to infants fed a soy-based formula.[292] These differences are associated with improved immune tolerance in the children fed the extensively hydrolyzed formula.[292] Similarly, children with peanut allergy who receive oral immunotherapy and subsequently develop immune tolerance have hypomethylation of the *FOXP3* locus compared to children performing allergen avoidance.[295] These studies provide compelling evidence that DNA methylation plays an important role in the development of food allergy, and that therapies that alter DNA methylation result in improved immune tolerance.

Obesity

Childhood obesity is associated with a pro-inflammatory state. This is linked to poor health outcomes, including the development of non-atopic asthma.[296,297] Immune cells from obese children demonstrate differential DNA methylation compared to non-obese children, and many of these differentially methylated genes are involved in immune pathways.[298–300] Obesity-associated asthma is a non-atopic Th1 polarized disease that is distinct from typical Th2 polarized atopic asthma.[301] Obese asthmatic children have hypomethylation of genes involved in T cell signaling and macrophage activation, including *CCL5*, *IL27*, *STAT1*, *IFNG*, *IL2RA*, *TBX21*, and *TGFB1*, in mononuclear cells compared to obese non-asthmatic children.[297] These findings are suggested to contribute to the non-atopic inflammation seen in obesity-associated asthma. Obesity and its related comorbidities are also associated with differences in microRNA expression.[302–304] Obese children have increased mononuclear cell miR-33a and miR-33b expression (involved in antiviral immunity) compared to non-obese children.[304] Obese adolescents with insulin resistance have increased peripheral blood miR-190b expression compared to obese adolescents without insulin resistance.[303] Additionally, obese children with endothelial dysfunction have increased plasma miR-365b-3p and decreased miR-125a-3p and miR-342-3p compared to obese children without endothelial dysfunction.[302] Childhood obesity alters immune cell epigenetic profiles, and these alterations are thought to contribute to obesity-related immune dysfunction and poor health outcomes.

Gastrointestinal diseases

Inflammatory bowel disease. Inflammatory bowel disease, including Crohn's disease and ulcerative colitis, develops in the context of disordered inflammation and a Th17 predominant phenotype.[305] Colonic tissue from pediatric patients with newly diagnosed ulcerative colitis demonstrates differential DNA methylation compared to tissue from healthy controls.[306] Several of these differentially methylated genes are associated with mucosal immunity and defense responses.[306] Numerous studies demonstrate differential microRNA expression in serum or intestinal tissue from pediatric patients with inflammatory bowel disease.[307–314] These differences are detailed in Table 4. Only a few of these microRNAs have been validated in multiple studies, and these include increased miR-142-3p, miR-146a, miR-21, miR-223, and miR-155 and decreased miR-124 in intestinal mucosa and increased miR-192 and miR-21 in serum from subjects with inflammatory bowel disease.[307–312,314] Commonly used treatment regimens for inflammatory bowel disease, including glucocorticoids and infliximab, alter microRNA expression, highlighting their role in disease pathogenesis.[315,316]

Celiac disease. Celiac disease is an autoimmune disease triggered by gluten ingestion that results in significant intestinal inflammation.[317] Pediatric patients with untreated celiac disease have increased serum miR-21 and decreased serum miR-31 compared to patients with treated celiac disease and healthy controls.[318] This points to a possible role for epigenetics in celiac disease symptomatology.

Intestinal failure/dysfunction. Environmental enteric dysfunction is an intestinal malfunction syndrome present in impoverished tropical areas that results in growth failure and is caused by T cell-mediated mucosal inflammation.[319] Duodenal tissue from children with environmental enteric dysfunction has DNA hypermethylation at genes involved in epithelial metabolism and barrier function (*TNXB*, *SERPINB5*) and hypomethylation of genes involved in immune responses and cell proliferation (*IFITM*, *PARP9*) compared to unaffected children.[319] Intestinal macrophages from children with other forms of intestinal failure have decreased miR-124 compared to children without intestinal failure.[320] miR-124 regulates intestinal macrophage activation, and may play a role in intestinal inflammation that is a hallmark of intestinal failure.[320]

Biliary atresia. Biliary atresia involves abnormal development of the liver bile ducts. Inflammation and scarring of the ducts are thought to contribute to disease development, but the exact etiology has yet to be determined.[321] Tregs from infants with biliary atresia have increased methylation of the *FOXP3* promoter compared to age-matched controls.[322] This is thought to contribute to impaired Treg suppressive function and exacerbate bile duct inflammation. Liver tissue from pediatric subjects with biliary atresia demonstrate increased miR-181 and miR-155 and decreased miR-29, miR-483, and miR-200 compared to healthy controls.[323,324] Downregulation of miR-155 reduces the incidence of biliary atresia in a rhesus monkey model, highlighting the role of epigenetics in disease development.[323]

Type 1 diabetes

Type 1 diabetes is caused by immune-mediated destruction of pancreatic beta cells, which results in insulin deficiency.[325] T cells, B cells, and monocytes from monozygotic twins with Type 1 diabetes demonstrate differential DNA methylation compared to their unaffected twin.[326,327] These differentially methylated sites involve immune and defense response genes, including several genes known to be associated with Type 1 diabetes (*HLA*, *INS*, *IL2RB*, *CD226*).[326] This differential methylation is not present in umbilical cord blood, suggesting that these DNA methylation changes are driven by postnatal environmental factors.[327] CD4+ T cells and Tregs from adolescents and young adults at risk for developing Type 1 diabetes (first-degree relative with type 1 diabetes, autoantibodies to at least two islet antigens) have differential microRNA expression compared to healthy controls.[328] This includes increased miR-181a and decreased miR-99b, miR-126, miR-33a, miR-194, and miR-340 in CD4+ T cells and increased miR-15a and decreased let-7c in Tregs.[328] These microRNAs have been proposed as useful biomarkers to identify disease risk. At the

Table 4. MicroRNA levels in pediatric inflammatory bowel disease.

MicroRNA	Tissue	Increased in	Compared to	Known function	Ref.
let-7g	Mononuclear cells	Inflammatory bowel disease following glucocorticoid treatment	Untreated inflammatory bowel disease	NF-κB signaling	316
let-7i	Colonic mucosa	Ulcerative colitis	Controls	TLR4 signaling	309
miR-100	Colonic mucosa	Non-diseased Crohn's disease mucosa	Controls	Tumor suppressor	307
Inflamed Crohn's disease mucosa					
miR-106a	Serum	Crohn's disease	Controls	Monocyte proliferation and differentiation	314
	Mononuclear cells	Inflammatory bowel disease following glucocorticoid treatment	Untreated inflammatory bowel disease		316
miR-122	Colonic mucosa	Non-diseased Crohn's disease mucosa	Controls	Liver homeostasis and hepatocyte innate immunity	311
		Non-diseased Crohn's disease mucosa	Inflamed ulcerative colitis mucosa		
miR-124	Colonic mucosa	Controls	Ulcerative colitis	Macrophage, dendritic cell and CD4+ T cell differentiation, inhibits inflammation	308,309
miR-125a	Colonic mucosa	Non-diseased Crohn's disease mucosa	Controls	Treg-mediated immune homeostasis	307
		Inflamed Crohn's disease mucosa			
		Ulcerative colitis			
			Ulcerative colitis	Inflamed Crohn's disease mucosa	
miR-126	Colonic mucosa	Non-diseased Crohn's disease mucosa	Controls	Pathogen-associated innate immune responses and Th2 differentiation	307
		Inflamed Crohn's disease mucosa			
		Ulcerative colitis			
miR-138-1	Colonic mucosa	Controls	Ulcerative colitis	NF-κB signaling	309
miR-140	Serum	Crohn's disease	Controls	T cell differentiation	314
miR-141	Colonic mucosa	Controls	Inflamed Crohn's disease mucosa	Tumor suppressor	307
			Ulcerative colitis		
		Non-diseased Crohn's disease mucosa	Inflamed Crohn's disease mucosa		
			Ulcerative colitis		
	Mononuclear cells	Inflammatory bowel disease following glucocorticoid treatment	Untreated inflammatory bowel disease		316
miR-142	Serum	Ulcerative colitis	Controls	B cell activation	310
		Crohn's disease			
	Mononuclear cells	Inflammatory bowel disease following glucocorticoid treatment	Untreated inflammatory bowel disease		316
miR-142-3p	Colonic mucosa	Controls	Non-diseased Crohn's disease mucosa	B cell activation	307
			Ulcerative colitis		
		Inflamed Crohn's disease mucosa			

Table 4 continued

MicroRNA	Tissue	Increased in	Compared to	Known function	Ref.
			Non-diseased Crohn's disease mucosa		
			Ulcerative colitis		
miR-142-5p	Colonic mucosa	Non-diseased Crohn's disease mucosa	Controls	B cell activation	307
		Inflamed Crohn's disease mucosa			
		Ulcerative colitis			
miR-144	Mononuclear cells	Inflammatory bowel disease following glucocorticoid treatment	Untreated inflammatory bowel disease	Cytokine responses	316
miR-146a	Colonic mucosa	Non-diseased Crohn's disease mucosa	Controls	Myeloid cell, B cell and T cell responses	307
		Inflamed Crohn's disease mucosa			
		Ulcerative colitis			
		Ulcerative colitis	Inflamed Crohn's disease mucosa		
		Inflamed ulcerative colitis mucosa	Non-diseased ulcerative colitis mucosa		311
		Inflamed Crohn's disease mucosa			
	Duodenal mucosa	Inflamed Crohn's disease mucosa	Non-diseased Crohn's disease mucosa		312
			Controls		
	Serum	Untreated inflammatory bowel disease	Inflammatory bowel disease following prednisone treatment		315
			Inflammatory bowel disease following infliximab treatment		
miR-146b	Colonic mucosa	Ulcerative colitis	Controls	B and T cell responses	309
	Serum	Untreated inflammatory bowel disease	Inflammatory bowel disease following infliximab treatment		315
	Mononuclear cells	Inflammatory bowel disease following glucocorticoid treatment	Untreated inflammatory bowel disease		316
miR-150	Colonic mucosa	Inflamed Crohn's disease mucosa	Controls	B and T cell differentiation	307
miR-155	Colonic mucosa	Inflamed ulcerative colitis mucosa	Non-diseased ulcerative colitis mucosa	NF-κB signaling, myeloid cell activation, T and B cell responses	311
		Inflamed Crohn's disease mucosa	Non-diseased Crohn's disease mucosa		
	Duodenal mucosa	Inflamed Crohn's disease mucosa	Controls		312
miR-15a	Ileocecal mucosa	Inflamed Crohn's disease mucosa	Non-diseased Crohn's disease mucosa	Macrophage differentiation and suppression of LPS-induced inflammation	313
			Ulcerative colitis		
			Controls		
miR-16	Serum	Crohn's disease	Controls	Macrophage differentiation and suppression of LPS-induced inflammation	314
miR-185	Colonic mucosa	Non-diseased Crohn's disease mucosa	Controls	Inhibits angiogenesis	307

Table 4 continued

MicroRNA	Tissue	Increased in	Compared to	Known function	Ref.
		Inflamed Crohn's disease mucosa			
miR-18a	Colonic mucosa	Non-diseased Crohn's disease mucosa	Controls	Th17 differentiation	307
		Inflamed Crohn's disease mucosa			
		Ulcerative colitis			
miR-192	Colonic mucosa	Controls	Ulcerative colitis	Cytokine responses	310
			Crohn's disease		
	Serum	Ulcerative colitis	Controls		
		Crohn's disease			
		Crohn's disease			314
miR-194	Colonic mucosa	Controls	Ulcerative colitis	Antiviral immunity	310
			Crohn's disease		
miR-195	Serum	Crohn's disease	Controls	Macrophage responses	314
miR-1973	Colonic mucosa	Ulcerative colitis	Controls	Cancer pathogenesis	309
miR-200b	Colonic mucosa	Controls	Ulcerative colitis	TLR4 signaling	310
			Crohn's disease		
miR-204	Colonic mucosa	Non-diseased Crohn's disease mucosa	Controls	Tumor suppressor	307
		Controls	Inflamed Crohn's disease mucosa		
		Non-diseased Crohn's disease mucosa	Inflamed Crohn's disease mucosa		
			Ulcerative colitis		
miR-20a	Colonic mucosa	Non-diseased Crohn's disease mucosa	Controls	Monocyte proliferation and differentiation	307
			Inflamed Crohn's disease mucosa		
			Ulcerative colitis		
	Serum	Crohn's disease	Controls		314
miR-21	Colonic mucosa	Non-diseased Crohn's disease mucosa	Controls	Myeloid cell proliferation and differentiation, B cell activation, Th17 differentiation, suppresses IL-12p35 expression	307
		Inflamed Crohn's disease mucosa			
		Inflamed Crohn's disease mucosa			
		Ulcerative colitis			309
	Serum	Ulcerative colitis	Controls		310
		Crohn's disease			
		Crohn's disease			314
miR-221	Colonic mucosa	Non-diseased Crohn's disease mucosa	Controls	Cytokine responses	307
miR-223	Colonic mucosa	Non-diseased Crohn's disease mucosa	Controls	Myeloid cell proliferation and differentiation	307
		Inflamed Crohn's disease mucosa			

Table 4 continued

MicroRNA	Tissue	Increased in	Compared to	Known function	Ref.
		Ulcerative colitis			
		Ulcerative colitis	Non-diseased Crohn's disease mucosa		
		Ulcerative colitis	Controls		309
miR-224	Colonic mucosa	Ulcerative colitis	Controls	T cell function	309
miR-24	Colonic mucosa	Ulcerative colitis	Crohn's disease	T cell function	310
			Controls		309
miR-29b	Mononuclear cells	Inflammatory bowel disease following glucocorticoid treatment	Untreated inflammatory bowel disease	IFN-γ signaling	316
miR-29c	Mononuclear cells	Inflammatory bowel disease following glucocorticoid treatment	Untreated inflammatory bowel disease	IFN-γ signaling	316
miR-30e	Serum	Crohn's disease	Controls	Innate immune responses	314
miR-31	Colonic mucosa	Non-diseased Crohn's disease mucosa	Controls	Treg function	307
		Inflamed Crohn's disease mucosa			
		Ulcerative colitis			
		Ulcerative colitis	Non-diseased Crohn's disease mucosa		
				Inflamed Crohn's disease mucosa	
	Mononuclear cells	Inflammatory bowel disease following glucocorticoid treatment	Untreated inflammatory bowel disease		316
miR-3133	Colonic mucosa	Controls	Ulcerative colitis	Tumor suppressor	309
miR-3173	Colonic mucosa	Ulcerative colitis	Controls	Tumor suppressor	309
miR-3182	Colonic mucosa	Ulcerative colitis	Controls	Cancer pathogenesis	309
miR-320a	Serum	Untreated inflammatory bowel disease	Inflammatory bowel disease following prednisone treatment	Macrophage responses	315
miR-34a	Colonic mucosa	Ulcerative colitis	Controls	T cell function	309
miR-3611	Colonic mucosa	Ulcerative colitis	Controls	Antiviral immunity	309
miR-363	Mononuclear cells	Inflammatory bowel disease following glucocorticoid treatment	Untreated inflammatory bowel disease	Cancer pathogenesis	316
miR-3646	Colonic mucosa	Ulcerative colitis	Controls	Cancer pathogenesis	309
miR-375	Colonic mucosa	Controls	Ulcerative colitis	Mucosal immunity	310
			Crohn's disease		
miR-378a	Colonic mucosa	Controls	Ulcerative colitis	Natural killer cell function	309
miR-378b	Colonic mucosa	Controls	Ulcerative colitis	Dendritic cell activation	309
miR-424	Colonic mucosa	Ulcerative colitis	Controls	Immune cell chemotaxis	309
miR-4284	Colonic mucosa	Controls	Ulcerative colitis	Cancer pathogenesis	309
miR-4286	Colonic mucosa	Controls	Ulcerative colitis	Cancer pathogenesis	309
miR-4323	Colonic mucosa	Controls	Ulcerative colitis	Cancer pathogenesis	309
miR-451a	Mononuclear cells	Inflammatory bowel disease following glucocorticoid treatment	Untreated inflammatory bowel disease	Innate immune responses	316

Table 4 continued

MicroRNA	Tissue	Increased in	Compared to	Known function	Ref.
miR-4772	Mononuclear cells	Inflammatory bowel disease following glucocorticoid treatment	Untreated inflammatory bowel disease	Treg function	316
miR-484	Serum	Crohn's disease	Controls	Antiviral immunity	314
miR-486	Serum	Untreated inflammatory bowel disease	Inflammatory bowel disease following prednisone treatment	Tumor suppressor	315
miR-548ak	Mononuclear cells	Inflammatory bowel disease following glucocorticoid treatment	Untreated inflammatory bowel disease	Antiviral response	316
miR-654	Mononuclear cells	Untreated inflammatory bowel disease	Inflammatory bowel disease following glucocorticoid treatment	Cancer pathogenesis	316
miR-7109	Mononuclear cells	Untreated inflammatory bowel disease	Inflammatory bowel disease following glucocorticoid treatment	Tumor suppressor	316
miR-873	Mononuclear cells	Inflammatory bowel disease following glucocorticoid treatment	Untreated inflammatory bowel disease	NF-κB signaling	316
miR-877	Colonic mucosa	Ulcerative colitis	Controls	Cytokine responses	309
miR-892a	Colonic mucosa	Ulcerative colitis	Controls	Cancer pathogenesis	309
miR-93	Serum	Crohn's disease	Controls	Hypoxia-induced immunosuppression	314
miR-96	Mononuclear cells	Inflammatory bowel disease following glucocorticoid treatment	Untreated inflammatory bowel disease	Tumor suppressor	316
miR-99a	Colonic mucosa	Non-diseased Crohn's disease mucosa	Controls	TGF-β signaling	307
		Inflamed Crohn's disease mucosa			
		Ulcerative colitis			
miR-99b	Colonic mucosa	Non-diseased Crohn's disease mucosa	Controls	Antigen presenting cell responses	307
		Inflamed Crohn's disease mucosa			
		Ulcerative colitis			

time of Type 1 diabetes diagnosis, several microRNAs are differentially expressed compared to nondiabetic children.[329–333] The only three microRNAs that have been validated in multiple studies are miR-24, miR-27a, and miR-27b, all of which are upregulated in peripheral blood of children with newly diagnosed Type 1 diabetes.[330,331,333] Different microRNA profiles have also been described based on severity of disease at the time of onset and time since disease diagnosis.[331–336] Table 5 highlights these differences. These studies demonstrate that immune cell epigenetic profiles are fluid during the progression of Type 1 diabetes and that different epigenetic mechanisms may play a role at different stages of the disease.

Rheumatologic diseases

Juvenile idiopathic arthritis. Juvenile idiopathic arthritis (JIA) is an immune-mediated disease that results in joint inflammation and damage.[337] Mononuclear cells from children with JIA have decreased expression of the DNA methyltransferases *DNMT1*, *DNMT3A,* and *DNMT3B* compared to healthy controls.[338] This suggests that DNA methylation may play a role in disease pathogenesis. Additionally, pediatric patients with JIA have increased plasma miR-155 and decreased plasma miR-204 compared to unaffected children.[339,340] These studies provide limited evidence that epigenetics contributes to JIA-associated pathology.

Juvenile systemic lupus erythematosus. Systemic lupus erythematosus (SLE) is chronic autoimmune disease that affects nearly every organ. Pediatric patients with SLE demonstrate differential DNA methylation in whole blood, CD4+ T cells, CD8+ T cells, B cells, and neutrophils compared to unaffected children.[341] Fifteen genes demonstrate hypomethylation in whole blood and across all purified cell lineages and are proposed as an SLE-specific DNA methylation signature. The hypomethylated genes include *IFI44L, MX1, PARP9, DTX3L, EPSTI1, IFI44, IFIT1, CMPK2, PLSCR1, DDX60, DDX58, USP18, RABGAP1L, FKBP5*, and *ISG15*.[341] Hypermethylation of the Treg locus *FOXP3* is also noted in whole blood from pediatric subjects with SLE, which may contribute to the autoimmune phenotype of the disease.[342] Pediatric patients with SLE also have decreased peripheral blood miR-155 and miR-181a compared to control children.[340,343] From these studies, it appears that epigenetic mechanisms contribute to autoimmunity that is a hallmark of SLE.

IgA vasculitis. IgA vasculitis is an immune-mediated vasculitis characterized by nonthrombocytopenic purpura, abdominal pain, and arthritis.[344] Children with active IgA vasculitis have significantly increased plasma levels of miR-33 and miR-34 and significantly decreased levels of miR-122 and miR-204 compared to children with inactive disease and healthy control children.[345] This suggests that microRNAs participate in active disease in IgA vasculitis.

Kawasaki disease. Kawasaki disease (KD) is a pediatric acute systemic vasculitis with an unclear etiology, although genetic and infectious factors are thought to contribute to disease development.[346] Subjects with KD demonstrate differential peripheral blood DNA methylation compared to healthy subjects and febrile non-KD subjects.[347–353] This includes hypomethylation of the *HAMP*, *FCGR2A*, *MMP-2*, *MMP-9*, *MMP-14*, *MMP-15*, *MMP-16*, *TLR1*, *TLR2*, *TLR4*, *TLR6*, *TLR8*, and *TLR9* loci in subjects with KD.[348,349,351–353] Administration of intravenous immunoglobulin (IVIG) is the standard of care for KD, and each of these gene loci demonstrate increased methylation following IVIG administration.[348,349,351–353] This is thought to be at least one mechanism by which IVIG dampens immune responses in patients with KD. Subjects with KD also demonstrate elevated serum miR-200c and miR-371-5p and altered Treg microRNA expression (increased miR-31, decreased miR-155 and miR-21) compared to healthy controls.[354,355] Interestingly, IVIG treatment also affects microRNA expression, and patients with KD demonstrate decreased Treg miR-31 and increased Treg miR-155 and miR-21 following IVIG administration.[355] Although the etiology of KD has yet to be clearly identified, epigenetics seems to at least be involved in the response to IVIG therapy.

Immune-mediated thrombocytopenia

Immune-mediated thrombocytopenia is characterized by isolated thrombocytopenia without alterations in other hematopoietic cell lines and is attributed to immune-mediated destruction of platelets and platelet precursors.[356] Polymorphism of the DNA methyltransferase gene *DNMT3B* is associated with an increased risk of childhood chronic immune thrombocytopenia.[357] It has also been demonstrated that children with primary immune thrombocytopenia have hypermethylation of the Treg locus *FOXP3* compared to unaffected children.[358] These studies link differential DNA methylation to disease pathogenesis in childhood immune thrombocytopenia. Pediatric patients with acute immune thrombocytopenia also have increased peripheral blood miR-302c-3p, miR-483-5p, miR-223-3p, miR-597 and decreased miR-544a compared to healthy controls and increased miR-302c-3p compared to pediatric patients with chronic immune thrombocytopenia.[359] This suggests that microRNAs may play a role in the pathogenesis of pediatric immune thrombocytopenia and may play a different role in acute and chronic forms of the disease.

Pulmonary diseases

Cystic fibrosis. Cystic fibrosis is a disease characterized by chronic respiratory infection and progressive respiratory insufficiency.[360] Children and young adults with a cystic fibrosis exacerbation have increased sputum miR-451a, miR-486-5p, and miR-17~92 cluster and decreased miR-19b, miR-223, and miR-27b-3p compared to patients without an exacerbation.[361,362] Many of these levels negatively correlate with lung function parameters, and could serve as useful biomarkers of respiratory status in patients with cystic fibrosis.

Bronchopulmonary dysplasia. Bronchopulmonary dysplasia (BPD) is a chronic lung disease related to prematurity. The causes of BPD are multifactorial and include oxygen toxicity, inflammation, and mechanical ventilation-induced lung damage.[363] Lung tissue from preterm infants with BPD demonstrate differential DNA methylation compared to preterm infants without BPD.[364] Differentially methylated genes are enriched in pathways involved in ErbB and nitric oxide signaling, both of which are associated with the development of BPD.[364]

From this section it is clear that epigenetics is involved in the pathogenesis of many childhood onset diseases. The contributions of DNA methylation and histone tail modifications to immune responses in childhood onset diseases are summarized in Fig. 6.

CONCLUSION

There is clear and compelling evidence that epigenetic mechanisms are involved in a broad array of biological processes related to immune development and immune health during childhood. Appropriate maturation of neonatal and pediatric immune responses is driven by epigenetic mechanisms, and a variety of prenatal, perinatal and postnatal exposures disrupt these epigenetic processes and contribute to poor health outcomes. Numerous pediatric-onset diseases also have an epigenetic component, and some commonly used treatment strategies influence immune epigenetic profiles and result in improvement or resolution of disease symptoms. The recent interest in the epigenetic regulation of pediatric immunity and immune-mediated diseases is encouraging, as this will likely lead to

Table 5. MicroRNA expression in pediatric-onset Type 1 diabetes mellitus.

MicroRNA	Tissue	Increased in	Compared to	Known function	Ref.
let-7d	Mononuclear cells	Controls	New onset Type 1 diabetes	Tumor suppressor	331
miR-10b	Plasma	Type 1 diabetes diagnosed 3–12 months prior	New onset Type 1 diabetes	Cancer pathogenesis	334
			Type 1 diabetes diagnosed greater than 1 year prior		
miR-122	Plasma	New onset Type 1 diabetes	Controls	Liver homeostasis and hepatocyte innate immunity	332
			Type 1 diabetes diagnosed over 10 years prior		
miR-1247	Mononuclear cells	Controls	New onset Type 1 diabetes	Cancer pathogenesis	331
		Mild onset Type 1 diabetes	Severe onset Type 1 diabetes (initial DKA)		
miR-125b	Plasma	Type 1 diabetes diagnosed 3–12 months prior	New onset Type 1 diabetes	TGF-β signaling, myeloid cell proliferation and differentiation and B cell activation	334
			Type 1 diabetes diagnosed greater than 1 year prior		
miR-126	Urine	Controls	Type 1 diabetes	Pathogen-associated innate immune responses and Th2 differentiation	335
miR-127	Mononuclear cells	Severe onset Type 1 diabetes (initial DKA)	Mild onset Type 1 diabetes	Antimicrobial immunity	331
miR-134	Mononuclear cells	Severe onset Type 1 diabetes (initial DKA)	Mild onset Type 1 diabetes	Antiviral immunity	331
miR-148a	Serum	New onset Type 1 diabetes	Controls	B cell function	330
miR-152	Serum	New onset Type 1 diabetes	Controls	Dendritic cell function	330
miR-17	Plasma	New onset Type 1 diabetes	Type 1 diabetes diagnosed 3-12 months prior	Myeloid cell proliferation and differentiation and B cell differentiation	334
		Type 1 diabetes diagnosed greater than 1 year prior			
miR-181a	Serum	New onset Type 1 diabetes	Controls	B and T cell differentiation and T cell responses	330
miR-185	Plasma	Type 1 diabetes diagnosed over 10 years prior	Controls	Inhibits angiogenesis	332
miR-192	Plasma	New onset Type 1 diabetes	Controls	Cytokine responses	332
miR-193b	Plasma	New onset Type 1 diabetes	Type 1 diabetes diagnosed over 10 years prior	Cytokine responses	332
miR-195	Plasma	Controls	Type 1 diabetes diagnosed over 10 years prior	Macrophage responses	332
miR-200a	Serum	New onset Type 1 diabetes	Controls	TLR4 signaling	330
miR-21	Plasma	Type 1 diabetes	Controls	Myeloid cell proliferation and differentiation, B cell activation, Th17 differentiation, suppresses IL-12p35 expression	335
	Urine	Type 1 diabetes	Controls		
miR-210	Serum	New onset Type 1 diabetes	Controls	B cell activation	330
	Plasma	Type 1 diabetes	Controls		335
	Urine	Type 1 diabetes	Controls		
miR-23a	Plasma	Type 1 diabetes diagnosed 12 months prior	New onset Type 1 diabetes	T cell differentiation	333
		Type 1 diabetes diagnosed 24 months prior			

Table 5 continued

MicroRNA	Tissue	Increased in	Compared to	Known function	Ref.
miR-23b	Plasma	Type 1 diabetes diagnosed 12 months prior	New onset Type 1 diabetes	T cell differentiation	333
miR-24	Serum	New onset Type 1 diabetes	Controls	T cell differentiation	330
		Controls	Type 1 diabetes		336
	Mononuclear cells	Severe onset Type 1 diabetes (initial DKA)	Mild onset Type 1 diabetes		331
	Plasma	Type 1 diabetes diagnosed 12 months prior	New onset Type 1 diabetes		333
		Type 1 diabetes diagnosed 24 months prior			
miR-25	Serum	New onset Type 1 diabetes	Controls	TGF-β signaling	330
miR-26a	Serum	New onset Type 1 diabetes	Controls	Inhibition of IL-6 expression	330
miR-27a	Serum	New onset Type 1 diabetes	Controls	Th2 differentiation	330
	Mononuclear cells	Severe onset Type 1 diabetes (initial DKA)	Mild onset Type 1 diabetes		331
miR-27b	Serum	New onset Type 1 diabetes	Controls	TGF-β signaling	330
miR-29a	Serum	New onset Type 1 diabetes	Controls	Common myeloid progenitor differentiation and response to bacteria	330
miR-30a	Serum	New onset Type 1 diabetes	Controls	Th17 differentiation	330
miR-30e	Plasma	New onset Type 1 diabetes	Type 1 diabetes diagnosed 3-12 months prior	Innate immune responses	334
		Type 1 diabetes diagnosed greater than 1 year prior			
miR-3180	Mononuclear cells	Controls	New onset Type 1 diabetes	Cancer pathogenesis	331
miR-3613	Mononuclear cells	Mild onset Type 1 diabetes	Severe onset Type 1 diabetes (initial DKA)	IFN-induced immune responses	331
miR-3652	Mononuclear cells	Controls	New onset Type 1 diabetes	Cancer pathogenesis	331
miR-375	Serum	Controls	New onset Type 1 diabetes	Mucosal immunity	329
miR-379	Mononuclear cells	Severe onset Type 1 diabetes (initial DKA)	Mild onset Type 1 diabetes	Tumor suppressor	331
miR-409	Mononuclear cells	Severe onset Type 1 diabetes (initial DKA)	Mild onset Type 1 diabetes	Tumor suppressor	331
miR-423	Plasma	New onset Type 1 diabetes	Type 1 diabetes diagnosed 3-12 months prior	Lysosome function	334
		Type 1 diabetes diagnosed greater than 1 year prior			
miR-445	Plasma	New onset Type 1 diabetes	Type 1 diabetes diagnosed over 10 years prior	Tumor suppressor	332
miR-451a	Mononuclear cells	Severe onset Type 1 diabetes (initial DKA)	Mild onset Type 1 diabetes	Innate immune responses	331
miR-455	Plasma	Controls	Type 1 diabetes diagnosed over 10 years prior	Tumor suppressor	332
miR-4668	Mononuclear cells	Mild onset Type 1 diabetes	Severe onset Type 1 diabetes (initial DKA)	TGF-β signaling	331
miR-4750	Mononuclear cells	Controls	New onset Type 1 diabetes	Unknown	331

Table 5 continued

MicroRNA	Tissue	Increased in	Compared to	Known function	Ref.
miR-487a	Mononuclear cells	New onset Type 1 diabetes	Controls	Tumor suppressor	331
		Severe onset Type 1 diabetes (initial DKA)	Mild onset Type 1 diabetes		
miR-497	Plasma	Type 1 diabetes diagnosed 3–12 months prior	New onset Type 1 diabetes	Tumor suppressor	334
			Type 1 diabetes diagnosed greater than 1 year prior		
miR-885	Mononuclear cells	Controls	New onset Type 1 diabetes	Cancer pathogenesis	331
miR-92b	Mononuclear cells	Mild onset Type 1 diabetes	Severe onset Type 1 diabetes (initial DKA)	Cancer pathogenesis	331
miR-93	Plasma	New onset Type 1 diabetes	Type 1 diabetes diagnosed 3-12 months prior	Hypoxia-induced immunosuppression	334
		Type 1 diabetes diagnosed greater than 1 year prior	Type 1 diabetes diagnosed 3–12 months prior		
miR-99a	Plasma	Type 1 diabetes diagnosed 3–12 months prior	New onset Type 1 diabetes	TGF-β signaling	334
			Type 1 diabetes diagnosed greater than 1 year prior		
	Mononuclear cells	Severe onset Type 1 diabetes (initial DKA)	Mild onset Type 1 diabetes		331

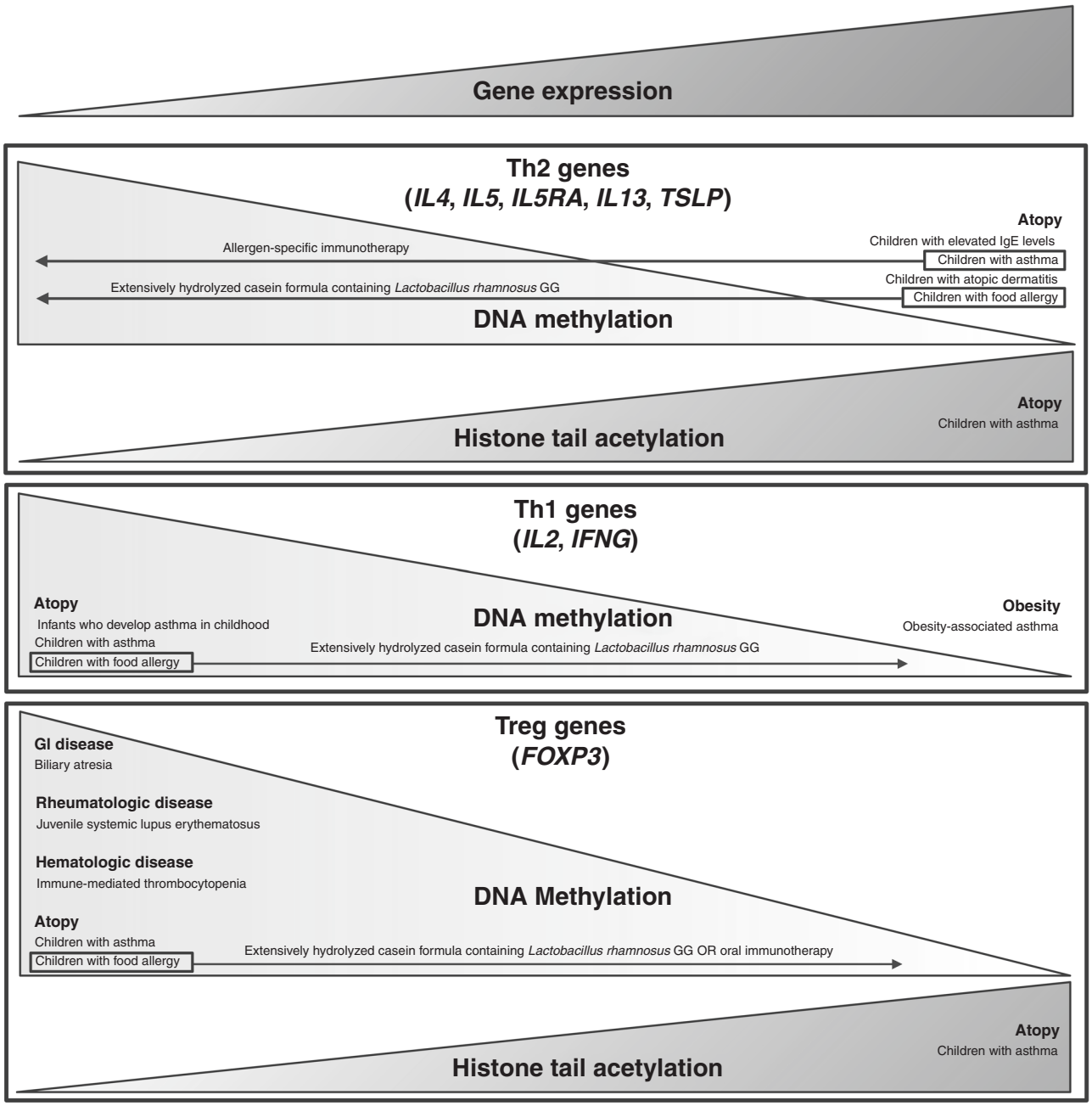

Fig. 6 Schematic representation of DNA methylation and histone tail modification changes in childhood onset diseases at key pro-inflammatory (Th1), atopic (Th2), and regulatory (Treg) immune genes. The impact of disease-specific therapies on DNA methylation at these sites is also depicted. Created with BioRender.com.

the identification of novel therapies and to significant improvements in health and quality of life at all stages of human development.

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The authors declare no competing interests.

ADDITIONAL INFORMATION

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