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Epigenetics in immune development and in allergic and autoimmune diseases

David Martino^{a,*,1}, Dörthe A. Kesper^{b,1}, Manori Amarasekera^{c,1}, Hani Harb^{b,1},
Harald Renz^{b,1}, Susan Prescott^{c,d,1}

^a Murdoch Childrens Research Institute, Department of Pediatrics, University of Melbourne, Australia

^b Institute of Laboratory Medicine, Pathobiochemistry and Molecular Diagnostics, Philipps University, Marburg, Germany

^c Childhood Allergy and Immunology Research, School of Paediatrics and Child Health, University of Western Australia, Australia

^d Telethon Kids Institute, Australia

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ABSTRACT

Epigenetic mechanisms such as DNA methylation, histone modification, and micro RNA signaling regulate the activity of the genome. Virtually all aspects of immunity involve some level of epigenetic regulation, whether it be host defense or in mediating tolerance. These processes are critically important in mediating dynamic responses to the environment over the life course of the individual, yet we are only just beginning to understand how dysregulation in these pathways may play a role in immune disease. Here, we give a brief chronological overview of epigenetic processes during immune development in health and disease.

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1. Introduction

Epigenetics is the study of heritable changes in transcriptional activity that are not caused by changes in DNA sequence. During immune cell development, epigenetic mechanisms mediate age-dependent and individual-specific changes in immune responses. Principally, this occurs via covalent modifications to DNA or DNA packaging proteins (such as histones), which result in alterations to the physical structure of chromatin and its amenability to transcription. These sequence-independent

changes are mitotically stable but reversible and tightly regulated over the life-course of the individual. Mounting evidence suggests that environmentally mediated disruption of the epigenetic program during early life might have lasting adverse consequences for immune development and immune disease. Moreover, novel data from animal models suggest that some of these disruptions might be transmissible across generations. This provides a new mechanistic framework for understanding the recent rise in complex immune diseases such as allergy and autoimmunity. Here, we provide a brief overview of the role of epigenetics in immune development and a summary of recent studies investigating epigenetic mechanisms of immune disease.

2. Epigenetic processes during hematopoietic development

During hematopoiesis, epigenetic mechanisms mediate developmental cascades, including the maintenance

Abbreviations: MC, mononuclear cells; Th, T-helper; DNAm, DNA methylation; ETS, environmental tobacco smoke; MTF, master transcription factor; TCR, T cell receptor; IL, interleukin; HSC, hematopoietic stem cells.

* Corresponding author at: Murdoch Childrens Research Institute, Royal Childrens Hospital, Flemington Road, Parkville, VIC 3055, Australia. Tel.: +61 9936 6621.

E-mail address: david.martino@mcri.edu.au (D. Martino).

¹ WUN International Inflammation Network (in-FLAME).

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of stem cell-like properties in progenitor cells, cell fate decisions, and lineage commitment. Modifications such as DNA methylation, typically associated with condensed chromatin and repressed genes, play a central role in the self-renewal capacity of hematopoietic stem cells (HSC). This is well demonstrated in knockout models of mice. Loss of the DNA-methyltransferase 1 (DNMT1) activity leads to the inappropriate differentiation of HSCs into myeloid progenitor cells (Broeske et al., 2009). In this context DNA methylation prevents the premature activation of differentiation programs in stem cells. Of equal importance is targeted de-methylation of genes by hydrolysis from TET family proteins. Mutations in TET proteins also appear to result in the skewing of myeloid cell fates (Moran-Crusio et al., 2011), suggesting that de-methylation of self-renewal factors might be equally important in maintaining pluripotency. Genes encoding polycomb group proteins, which confer repressive H3K27 methylation modifications to histones, are required for correct hematopoietic development. Enforced expression of polycomb repressive complex 1 (PCR1) genes augments self-renewal in HSC (Konuma et al., 2010), whilst mutations in PCR1 components are associated with the depletion of self-renewal capacity over time (Iwama et al., 2004).

Upon lineage commitment, epigenetics processes are closely associated with the repression of stem cell-specific genes, and the expression of genes important for differentiation. In progenitor cells, KIR genes (killer cell Ig-like receptor) exhibit characteristics of epigenetic repression including repressive histone tail modifications and densely methylated chromatin. Differentiation along the natural killer cell (NK) lineage is accompanied by the acquisition of H4K8 acetylation (active) and loss of H3K9 (repressive) histone marks at this locus (Santourlidis et al., 2008). In B-lymphocyte development, the *MB-1* gene (Cd79a) encodes the Ig α component of the B-cell receptor. The transcriptional activation of *MB-1* brought about by extrinsic transcription factors drives de-methylation at the *MB-1* promoter and concomitant gene expression during the course of B cell development (Ramirez et al., 2008). The regulation of double positive thymocyte differentiation into CD4 and CD8 subsets occurs via the transcription factor ThPOK interactions with lineage-specific silencers or through *RUNX3* proteins (Cao et al., 2010), resulting in epigenetic regulation of the *CD4* and *CD8* loci (Rui et al., 2012). Disruptions in these processes can give rise to pools of malignant cells with dysregulated proliferation and differentiation potential. Mutations in DNA methyltransferase enzymes, TET proteins or polycomb repressive complex subunits are frequently observed in pediatric and adult leukemia (Cullen et al., 2014). Thus, correct regulation of the epigenetic machinery is essential to normal hematopoietic development and to the maintenance of blood cell phenotypes.

MicroRNA (miRNA) signaling plays an equally important role in cellular immune development. These small RNA molecules can regulate gene expression by targeted degradation of mRNA transcripts and direct inhibition of protein translation (Gracias and Katsikis, 2011). Profiling studies have revealed differential expression of miRNA species during TLR-induced signaling in human monocytes

(Taganov et al., 2006), differentiation of monocyte-derived dendritic cells (Hashimi et al., 2009), and granulocyte development and function (Johnnidis et al., 2008). Deletion of dicer in T-cells (a protein complex critical for miRNA biogenesis) results in impaired T-cell development and survival (Cobb et al., 2005). T-cell homeostasis also appears to involve miRNAs as targets of FoxP3, which are important for the suppressor function (Huang et al., 2009). The overall picture therefore suggests that epigenetic marks might provide finely tuned control of hematopoietic developmental cascades.

3. The epigenetic profile of the neonatal immune system

The neonatal immune system involves a number of unique immunological phenomena specific to this period of immune development (Adkins, 2013). During pregnancy, adaptive changes in immunity are required to prevent harmful cell-mediated immune responses toward the developing fetus. Underpinning this phenomenon are molecular epigenetic processes operative in a range of cell types. At the fetomaternal interface, significant hypomethylation of *FOXP3* is observed in fetal cytotrophoblasts in placental tissue (Prescott et al., 2011). Many immune response genes are hypermethylated in fetal macrophage cells, whilst macrophage activation genes are hypomethylated in maternal decidual macrophages (Kim et al., 2012). These mechanisms are thought to be critical in maintaining an anti-inflammatory environment at the fetomaternal interface.

In both humans and mice Th1 responses *in vivo* and *in vitro* are broadly suppressed in favor of Th2 immunity in the neonatal period. The *IFNG* gene, the prototypical Th1 cytokine gene is hypermethylated in resting neonatal CD4⁺ cells compared with adult CD4⁺ cells (White et al., 2002), and this likely underpins reduced IFN- γ response capacity frequently observed in neonatal immune responses. Similarly, chromatin accessibility at the *TBX21* locus, the master regulator of Th1 lineage commitment is attenuated in neonatal CD4⁺ cells relative to adult cells (Kaminuma et al., 2009). Together, this suggests that neonatal immune responses might be epigenetically biased against robust Th1-mediated immunity. Further evidence of this has been demonstrated in monocyte-derived dendritic cells (DC), which have impaired capacity to secrete the pro-Th1 cytokine IL-12 in the neonatal period (Goriely et al., 2004). Thus, the unique features of neonatal immune responses are mediated, at least in part, via epigenetic processes and these are likely critical in mammalian adaptive immunity during gestation.

4. Epigenetics in perinatal and postnatal immune development

The perinatal period surrounding birth is a crucial time for the induction of immunological tolerance. It is well accepted that both endogenous and exogenous factors drive this late-stage maturation of the immune system. Exposures during this period may be 'prime-time' for programming disease risk later in life (Fall, 2012). The

well-studied factors influencing immune system maturation and later disease risk include microbial contact, nutrition and environmental pollutants, and there is evidence that the effects of these exposures may be mediated via the epigenome.

Bacterial exposures are particularly important for driving the maturation of the immune system. After birth, exposure to a diverse range of microorganisms and establishment of the microbiota promotes necessary up-regulation of Th1 immune responses. In mice it has been shown that prenatal administration of Gram-negative bacteria results in histone H4 acetylation at the *IFNG* gene, and associated up-regulation of IFN- γ production in the offspring (Brand et al., 2011). This is an example of proof of principle for the hygiene hypothesis as the F1 progeny mice were protected from the asthmatic phenotype induced by allergen challenge. Importantly, this is one of many studies in animal models that suggest that prenatal exposures might alter the epigenetic status of genes in the developing fetus. The evidence for these transgenerational effects in humans is growing. A recent study suggests that prenatal environmental factors (maternal allergy, maternal cytokine production, exposure to tobacco smoke) might modify DNA methylation of the *FOXP3* locus in cord blood (Hinz et al., 2012). Additionally, maternal nutrition (Loke et al., 2013; Waterland et al., 2006) and exposure to environmental pollutants (Koestler et al., 2013) have been identified as factors that can alter epigenetic profiles in the developing offspring. These mechanisms may underpin the observed differences in immune function present at birth in children predisposed to allergic diseases in early childhood (Smith et al., 2008; Tulic et al., 2011). Whilst this has yet to be fully explored, the emerging evidence suggests that maternal exposures might potentially modify fetal epigenetics at key immune genes. The extent to which this modifies immune phenotypes and immune function remains a key question.

In postnatal development neonatal responses must undergo environment-driven maturation to achieve full maturity (Prescott et al., 1999). Developmental changes in immune function are concomitant with epigenetic changes in immune cell genomes, mediated via external signals from the environment. Correct mediation of these signals via the epigenome is likely crucial in preventing the development of inappropriate immune responses.

Using a genome-wide approach we have previously characterized the longitudinal changes in DNA methylation of mononuclear cells (MC) from birth to 5 years of age (Martino et al., 2011). In this experiment we repeatedly measured DNA methylation in the same group of individuals at birth, 12 months, 2.5 years, and 5 years using microarrays. In the first year of life we observed widespread and substantial changes in DNA methylation levels at cell signaling and communication pathways initiated at the plasma membrane, a finding likely to reflect innate and adaptive immune pathways responsive to the new antigen-rich environment (Monteleone et al., 2006). In the postnatal period (1–5 years) the rate of change of DNA methylation was markedly reduced compared with the first 12 months after birth. Differential methylation in postnatal development was primarily enriched at genes

regulating immune-cell receptor recombination, the patterning of effector responses, complement and immunoglobulin development. These are likely to reflect the highly individualized antigenic experience of the adaptive immune system. In the postnatal period the epigenome, therefore, appears to be highly responsive to environmental exposures that signal through immune receptor pathways. The extent to which these pathways stably underscore variation in host immune responses at the individual level, or at the tissue or population level, is yet to be fully explored.

5. Epigenetic associations with allergic and autoimmune diseases

Allergic and autoimmune diseases like asthma, multiple sclerosis (MS) or rheumatoid arthritis (RA) develop because of an inappropriate immune response toward harmless external allergens (allergic disease) or against auto-antigens of the body (autoimmune disease). Whilst part of the underlying problem with these complex immune diseases is a genetic risk, genetic factors alone fail to completely explain the increasing prevalence of disease, implicating nongenetic components of disease (Martino and Prescott, 2013). Epigenetic mechanisms have more recently come under investigation as important disease components since these mechanisms are responsive to the environment and can modify disease risk in a manner analogous to polymorphisms (Liu et al., 2013).

To identify epigenetic mechanisms and environmental factors controlling the immune response in allergic and autoimmune disease several studies have been conducted in humans and in supportive animal models (Brand et al., 2012; Hollingsworth et al., 2008). Epigenetic analysis of monozygotic twins discordant for these diseases has revealed differential epigenetic programming of immune cells, as well as tissue-derived cells in target organs. Genome-wide or candidate gene-specific case-control studies have identified additional loci that undergo differential epigenetic modification (Table 1) (Carlsen et al., 2013; Graves et al., 2013; Liu et al., 2013; Luo et al., 2014; Runyon et al., 2012; Stefan et al., 2013; Stefanowicz et al., 2012).

The environmental factors influencing the disease susceptibility of allergic and autoimmune disease by epigenetic mechanisms include diet, environmental pollutants, and drugs. They can act upon several epigenetic mechanisms like DNA methylation and histone modifications. Especially well analyzed in this regard are the dietary intake of methyl donors and the exposure to environmental tobacco smoke (ETS). Dietary methyl donor intake influences primarily DNA methylation and modifies the risk for asthmatic disease as well as for autoimmune disease such as MS (Sharma and Litonjua, 2013; Zhu et al., 2011). ETS seems to have effects on DNA methylation and on histone modifications and is associated with an increased risk for developing asthma or MS (Hedström et al., 2011; Treyster and Gitterman, 2011). Altered methylation has been reported at the *ACSL3* gene promoter (measured in cord blood) in association with maternal airborne polycyclic aromatic hydrocarbon (PAH)

Table 1

Genome-wide or candidate gene-specific studies describing additional loci that undergo differential epigenetic modification.

Disease	Study type	Outcome	References
Atopic dermatitis (AD)	Case–control	The <i>TSLP</i> promoter exhibits hypomethylation in keratinocytes in patients with AD compared with healthy controls.	Luo et al. (2014)
Asthma	Monozygotic twins	Isolated Tregs and T-effector cells from discordant twins exhibit impaired Treg and T effector function, decreased Foxp3 and IFN γ expression accompanied by increased DNA methylation of the <i>FOXP3</i> and <i>IFNG</i> locus in the asthmatic twin.	Runyon et al. (2012)
Asthma	Case–control	Methylation of 1505 CpG loci was analyzed in airway epithelial cells (AECs) and PBMCs from healthy, atopic, and asthmatic children. Differential DNA methylation was identified exclusively in AECs. Higher DNA methylation of the <i>STAT5A</i> was accompanied by decreased gene expression.	Stefanowicz et al. (2012)
Multiple sclerosis	Case–control	Genome-wide methylation analysis in CD4+ T cells revealed differential methylation at the <i>HLA-DRB1</i> locus.	Graves et al. (2013)
Rheumatoid arthritis	Case–control	Genome-wide analysis of blood cells from 354 RA patients and 337 controls revealed differential methylation of two clusters within in the MHC locus that potentially mediates genetic risk.	Liu et al. (2013)
Systemic lupus erythematosus (SLE)	Case–control	Determination of 45 miRNAs revealed 7 differentially expressed miRNAs in plasma from patients in comparison to healthy controls. A 4-miRNAs signature was diagnostic for SLE.	Carlsen et al. (2013)
Type 1 diabetes	Monozygotic twins	Analysis of the DNA methylation of 27,578 CpGs in lymphocytes of three discordant and six concordant twin pairs identified 88 differential methylated sites in discordant twins.	Stefan et al. (2013)

exposure and parent-reported asthma in children prior to age 5 ($n=56$) (Perera et al., 2009). A follow-up study reported that maternal PAH exposure was associated with increased promoter methylation of *IFNG* in cord blood DNA (Tang et al., 2012). In vitro exposure of Jurkat and lung cell adenocarcinoma cell lines to non-cytotoxic doses of PAH confirmed increased *IFNG* methylation and reduced expression of *IFNG*. These findings support the potential role of altered fetal programming mediated via epigenetics in response to maternal environmental exposures.

A key challenge in disease-association epigenetic studies involves establishing causality. Given that epigenetic modifications are dynamic and change over time, and vary in a cell- or tissue-specific manner, establishing causality is not straightforward. Epigenetic variation could arise as a cause or a consequence of disease pathogenesis. An example of this could include long-term alterations in the composition of blood cells in immune diseases. In order to conclusively demonstrate a functional role of epigenetics in the disease process, it will be crucial to determine whether variation exists prior to disease onset (Michels et al., 2013). Other approaches to addressing this issue include the analysis of multiple tissues or cell types from cases and controls. Identifying disease-associated epigenetic variation in multiple tissues of different lineages implies that these marks were likely established very early in development, prior to disease manifestation (Rakyan et al., 2011). Recent technological developments have now facilitated the study of epigenetics on a genome scale. These epigenome-wide association studies (EWAS) are most commonly conducted at the level of DNA methylation, but other epigenetic marks are also amenable to genome-scale profiling. Care must be

taken to account for potential genetic effects on epigenetic modifications such as DNA methylation. Polymorphisms at CG dinucleotides can abolish the potential for methylation to occur, which could be mistakenly interpreted as a long-term stable epigenetic phenomenon. Current-generation EWAS study designs recommend obtaining sequence-specific data as well as screening multiple tissues at multiple time points (Ke et al., 2013). These studies are therefore incredibly complex but likely to reveal highly meaningful insights into human population epigenetics.

6. Summary

The science of epigenetics provides an exciting new mechanistic framework for understanding complex gene–environment interactions and their effects on immune development and disease. The molecular mechanisms required for transmitting the memory of environmental exposure and programming disease risk are now being described in these studies. The remaining challenge includes establishing a causal role for these epigenetic effects in modifying the normal course of immune development. Given the dynamic and fluctuating nature of the epigenome over time, these challenges can only be met with appropriate study designs in well-controlled experimental settings.

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