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# DNA methylation in the immune system

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Each nucleated somatic cell in our bodies contains a roughly identical complement of genetic material. Yet, some cells exhibit the specialized features of muscle and others skin; some cells transmit neural impulses and still others fight viral infections. This diversity of cell fate and function results from unique combinations of transcriptional activators and repressors that regulate patterns of gene expression and silencing throughout development.

For *trans*-factors to exert their influence they must have access to relevant gene control elements located in chromatin. This is not a straightforward proposition as interphase chromatin is highly ordered, with nonreplicating DNA intimately packaged into repeating, histone-rich nucleosomes. One feature of chromatin that supports *trans*-factor-induced cell specification and function is a dynamic nucleosome structural configuration, which may be altered to provide or limit access to specific DNA regulatory regions. The elements controlling heritable changes in chromatin configuration and gene expression patterns without altering the primary DNA sequence itself are termed "epigenetic" modifications. These include changes in nucleosome positioning, histone content, and patterns of covalent amino-terminal histone modification that form a "histone code" for effector protein recruitment and DNA methylation [1,2].

Determining the signals and order of changes that dictate dynamic transitions in chromatin structure between active and silent states is a current challenge in epigenetic research. Some studies in plants, in filamentous fungi, and in mammalian X chromosome inactivation indicate that changes in interphase chromatin are initiated by histone modifications [3–6]. Subsequent recruitment of specific effector proteins causes either reduced DNA methylation levels in locus activation or dense DNA methylation in locus silencing. However, other studies have also shown that DNA methylation can precede and direct histone modifications to effect changes in chromatin configuration [7]. Furthermore, de novo or maintenance DNA methylation following DNA replication likely precedes histone recruitment and DNA encasement in chromatin and may dictate subsequent histone modifications [8]. Clearly, the linear progression from histone alterations through *trans*-factor recruitment to subsequent adjustments in DNA methylation levels resulting in chromatin accessibility or closure is an oversimplification of the epigenetic changes that regulate gene expression and remains to be established in higher eukaryotes.

The discovery of DNA methylation in mammals was reported in calf thymus DNA in 1948, long before investigations of additional epigenetic phenomena that control the chromatin template [9]. Over time, DNA methylation studies have greatly benefited from work with immune system cells, which offer key advantages including easy isolation, well-defined developmental stages and lineage relationships, and established patterns of gene expression and silencing for many immune-specific genes. DNA methylation is also increasingly recognized for its prominent role in controlling diverse immune processes including hematopoietic cell development and lineage decisions, immune competence, antigen receptor repertoire, antigenic reactivity, autoimmunity, viral life cycle, tumor monitoring, hematopoietic neoplasms, and age-related changes in immune status. Combined, the advantageous features and broad impact that regulation of the chromatin template has on immune cells continue to provide fertile territory for studying DNA methylation.

The signals that recruit the DNA methylation (and demethylation) machinery to specific chromosomal locations

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are under active investigation in immune and other cell types. In mammals DNA methylation occurs on carbon-5 of the cytosine base. Targeted cytosines occur mainly in CG dinucleotides, although rare non-CG DNA methylation has also been reported [10–14]. Not all CG dinucleotides are equivalent and methylation is typically excluded from so-called "CG-rich islands" found in the promoter sequences of many constitutively expressed "housekeeping" genes [2,15,16]. Methyl groups themselves are small and hidden within the internal CG base-pairing region of the DNA double helix. Not surprisingly, the conformational difference in DNA containing or lacking a cytosine methyl group is minimal [17]. Yet, this difference is sufficient for recognition by specialized methyl DNA binding proteins, around which typically repressive, closed chromatin is assembled and gene silencing is established through corecruitment of transcriptional repressor complexes [18,19]. While the enzyme(s) that actively removes methylcytosine remains elusive or controversial, much is known about the de novo (DNMT3a and DNMT3b) and maintenance (DNMT1) DNA methyltransferase enzymes that add methyl groups to cytosines following DNA replication [20–23]. Here, Pradhan and Esteve discuss the enzymology of methyl group addition to DNA and DNMT expression within the immune system.

Mutation of DNMT3b disrupts the usual pattern of cytosine methylation and causes the only naturally occurring human anomaly associated with defects in a DNA methyltransferase gene, the ICF syndrome [24]. As described by Ehrlich, this chromosome breakage disease features agammaglobulinemia or combined immunodeficiency, centromeric region genome instability, and facial anomalies as a diagnostic triad. In recent studies, a hypomorphic allele of Dnmt1 in mice caused DNA hypomethylation, genomic instability in mature or activated lymphocytes, and an increased incidence of lymphoma formation [25,26]. Combined, findings in this model and in the ICF syndrome clearly demonstrate the deleterious effects of reduced or absent DNA methyltransferase expression in the immune system.

Early lymphocyte development is characterized by sequential antigen receptor V(D)J gene segment rearrangements during the assembly of functional antigen receptor genes. How particular gene segments that make up mature immunoglobulin or T cell receptor molecules are chosen for recombination has remained an active area of investigation for many years. Yancoupolos and Alt proposed long ago that accessibility of the recombinase machinery to relevant gene segment control regions, such as recombination signal sequences, dictates which segments rearrange to form productive antigen receptors [27,28]. Regulation of antigen receptor gene segment accessibility is summarized by Inlay and Xu with the accumulating evidence indicating that epigenetic alterations including DNA methylation and demethylation figure prominently in determining the mammalian B and T cell antigen receptor repertoire.

Overall DNA methylation patterns are established in early embryogenesis following erasure after gamete fusion and remethylation of the genome after zygote implantation in the uterus [29]. Less established, however, is the active or passive role(s) assumed by DNA methylation in controlling more developmentally advanced lineage decisions and cellular physiology. As discussed by Fitzpatrick and Wilson, dynamic DNA methylation and demethylation regulate T lineage development and differentiation, activation, and repression of CD4 and CD8 coreceptor molecules, T cell cytokine (IL-2, IL-4, and IFN-$\gamma$) expression, and TH1 versus TH2 helper T cell polarization. Additional studies summarized by van den Elsen and colleagues highlight the critical role of DNA methylation in regulating major histocompatibility complex and class II transactivator gene expression in antigen-specific immune responses, tumor development, and immune surveillance. Clearly, DNA methylation controls key aspects of peripheral immune system effector cell development and activity.

The vast majority of mammalian DNA consists of silent heterochromatic, centromeric, and pericentromeric chromatin in which integrated retroviruses, highly repetitive sequences (e.g., *Alu*, LINE and SINE elements), and potentially active transposable elements are found. Most DNA methylation is associated with these silent regions, where it acts to permanently suppress the expression and movement of these endogenous and parasitic genetic elements. Interestingly, the genome of episomally replicating Epstein–Barr virus (EBV) is also methylated. As discussed by Tao and Robertson, EBV makes use of DNA methylation to persist in different states of latency within infected B cells. Viral proteins that are highly immunogenic are kept silent by reversible latency-associated viral promoter region methylation, facilitating avoidance of immune detection by antiviral cytotoxic T cells [30–32]. Lytic cycle activation may be induced naturally or with drug treatments that demethylate lytic cycle-associated viral promoters. This occurrence provides a potential therapeutic strategy in which demethylating agents may be used to induce production of immunogenic viral proteins, activating a robust CTL response against EBV-infected malignant cells.

Interestingly, the immune system recognizes CG dinucleotides that lack DNA methylation as foreign, which may activate innate and adaptive immunity [33,34]. Unlike mammals, bacterial DNMTs methylate non-CG cytosine (and adenine) sequences. With the exception of small amounts of nonmethylated CG from CG island-containing promoters, host immune systems are overwhelmingly exposed and likely tolerized to methyl-CG sequences from pathologic and normal cell death. Numerous synthetic oligodeoxynucleotides (ODNs) containing unmethylated CG dinucleotides have been screened for immune-modulating activities. Two structural classes of ODN, K-type and D-type ODNs, stimulate distinct primate primary blood mononuclear cell types. As discussed by Verthelyi and Klinman, the use of these classes of synthetic ODNs holds promise as

vaccine adjuvants and as potential therapy for treating cancer, allergy, and asthma.

Aberrant levels and patterns of DNA methylation stimulate T cell autoreactivity and autoimmunity. As discussed by Richardson, exogenous inhibitors of T cell methylation, such as treatments with procainamide or hydralazine, result in a lupus-like disease in animals that mirrors many key aspects of idiopathic lupus in humans. In vitro, these drug treatments cause abnormal expression of genes with potential roles in autoimmunity and reduce the activation threshold for human and murine $CD4^+$ T cells. Additionally, hypomethylation of Th cells in vivo causes increased anti-DNA antibody production through B cell stimulation and lethal interactions for macrophages and perhaps additional antigen-presenting cells. These pleiotropic effects on immune recognition and reactivity seem due, in part, to altered levels of cell adhesion molecules and decreased clearance of apoptotic materials by reduced numbers of macrophages. DNA hypomethylation may contribute to additional T-cell-mediated autoimmune diseases, including rheumatoid arthritis in humans and scleroderma or Sjogren's syndrome models in mice. Combined, these findings indicate a strong link between reduced DNA methylation and altered immune self-recognition and predict increased autoimmune disease with aging and erosion of "younger" DNA methylation patterns.

Somehow, the usual pattern of DNA methylation outside CG islands is reversed in cancer, causing hypermethylation of CG-rich promoters and overall genomic hypomethylation, resulting in decreased genomic stability [25,26,35]. The mechanism(s) for upsetting this balance is unknown but current theories include an abnormal spreading from normal methylation centers or aberrant seeding and spread from a nidus of abnormal methylation. Neoplastic patterns of promoter methylation can be as harmful as gene silencing mutations in situations in which normally expressed tumor suppressor genes are silenced by chromatin closure and compaction. As discussed by Esteller, this aberrant methylation process is nonrandom and specific gene hypermethylation appears associated with specific immune and nonimmune malignancies [36,37]. Implications for recurring patterns of aberrant DNA methylation associated with specific malignancies include potential new insights into pathways leading to specific types of cancer and the possibility for improved classification, diagnosis, therapies, and outcome predictions.

Drug-induced correction or blockade of altered DNA methylation patterns is an exciting potential therapy for cancer. Here, Leone and colleagues describe the rationale and use of inhibitors of DNA methyltransferase activity in clinical trials for acute myeloid leukemia, myelodysplastic syndrome, and treatment-induced secondary leukemias. These lesions are prone to specific, tumor suppressor gene hypermethylation and aberrant gene silencing. 5-Azacytidine and structural analogues are potent DNA methyltransferase inhibitors that have been used safely and with efficacy, resulting, for example, in high response rates and improved survival for MDS patients [38,39]. Ongoing trials with newer agents and combinatorial drug regimens could further improve the outcome for patients with these and additional hematologic malignancies.

Finally, as we age our innate and adaptive immune systems may falter, leaving us increasingly susceptible to infectious, malignant, and possibly autoimmune diseases. Paralleling this decline in immune function, aging cells subtly change their patterns of DNA methylation. Overall, cells and tissues become hypomethylated while selected genes become progressively hypermethylated and, potentially, permanently silenced. This pattern of changes is strikingly reminiscent of the changes that are observed in malignant cells, with general genome hypomethylation and characteristic patterns of selective gene hypermethylation and silencing. As discussed by Issa, studies into the effects on immune system function with aging due to changes in DNA methylation patterns are in their infancy. Hypomethylation results mainly from decreased levels in pericentromeric repeat and parasitic genome elements, where most methylation is usually found. Causes for age-related methylation changes remain unknown and the effect on immune cells is mainly speculative.

In summary, one thing seems abundantly clear: We are only beginning to scratch the surface of the roles that normal DNA methylation plays in immune system development and function and the roles that abnormal DNA methylation plays in pathologic states, such as leukemia and autoimmunity. We hope you will enjoy this timely and provocative special collection of papers on the roles of DNA methylation in the immune system.

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