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REVIEW

Epigenetic alterations and autoimmune disease

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Recent advances in epigenetics have enhanced our knowledge of how environmental factors (UV radiation, drugs, infections, etc.) contribute to the development of autoimmune diseases (AID) in genetically predisposed individuals. Studies conducted in monozygotic twins discordant for AID and spontaneous autoimmune animal models have highlighted the importance of DNA methylation changes and histone modifications. Alterations in the epigenetic pattern seem to be cell specific, as $CD4^+$ T cells and B cells are dysregulated in systemic lupus erythematosus, synovial fibroblasts in rheumatoid arthritis and cerebral cells in multiple sclerosis. With regard to lymphocytes, the control of tolerance is affected, leading to the development of autoreactive cells. Other epigenetic processes, such as the newly described miRNAs, and post-translational protein modifications may also be suspected. Altogether, a conceptual revolution is in progress, in AID, with potential new therapeutic strategies targeting epigenetic patterns.

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Introduction

Autoimmune diseases (AID) include over 80 different disorders that cumulatively affect up to 10% of the population and represent the third leading cause of morbidity in Western countries.[1] AID have a broad range in organ-specific diseases such as diabetes type 1, multiple sclerosis (MS) and primary biliary cirrhosis and in systemic non-organ diseases such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA) and Sjögren's syndrome (SS). The onset of AID is related to a breakdown of tolerance, with the immune system unable to discriminate between self- and non-self-antigens. Genetic and environmental factors have been implicated, although the exact mechanisms causing AID remain poorly understood.[2]

Among the genetic risk factors associated with AID,[3] the most important is related to the antigen presentation process, with a particularly significant contribution from the major histocompatibility complex class II system. In addition, minor genetic risk factors have been characterized, revealing the importance of additional processes including lymphocyte activation, the complement pathway, the process of apoptosis, the clearance of immune-complexes and genes acting as key regulators of epigenetic control (Table 1). From these studies, it is now well established that the genetic background is important but not sufficient to develop an AID. This assertion has been supported by analysis of the concordance rate between monozygotic twins (MT) compared with dizygotic twins (DT) or relatives.[4] Indeed, the concordance rate in MT reaches '25–60%', which is higher than the ~5% observed in DT and relatives, but not close to '100%'. This then confirms a requirement for other endogenous and exogenous factors.

Epigenetics is a novel area of research in AID, which can be defined as reversible and potentially heritable changes in gene expression that do not affect the genetic code.[5] In organ- and non-organ-specific AID, the epigenetic contribution is supported by several observations. First, AID is found predominantly in women, it increases with age and can be exacerbated by external factors (infection, exposure to sunlight, drugs, etc.) or internal factors (sex, pregnancy, stress). Second, among the approximately 100 drugs that have been reported to induce AID, most of them induce epigenetic modifications as observed with hydralazine, procainamide and isoniazid.[6] Third, at the cellular level, epigenetic dysregulations in AID are related to modifications in gene transcription (DNA methylation and histone modifications), mRNA transcript dysregulation (micro-RNA) and protein post-translational modifications. With regard to the relevance of the epigenetic process in AID, the present review will focus on the immune system and summarize recent data showing that epigenetic control of B- and T-lymphocyte regulation is defective in patients with AID.

Chromatin accessibility in AID

The DNA methyl transferases (DNMT) catalyze the transfer of a methyl group from the methyl donor S-adenosyl-L-methionine (SAM) to the 5′carbon of the cytosine ring in cytosine guanine (CpG) pairs. Among the five known DNMTs, DNMT1 methylates hemi-methylated substrates,

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Table 1. *Genetic risk factors associated with autoimmune diseases*[6,72]

Function	Genes	Associated diseases
Antigen presentation	CMH-I, CMH-II	SLE, RA, diabetes
Complement pathway	C1q, C_2, C_{4A}, C_{4B}, MBL2	SLE
Lymphocyte activation	BANK1/BLK, Lyn, PTPN22	SLE, RA, diabetes, SSc
Apoptosis clearance	CRP, FEGR2, FCGR3	RA
Apoptosis regulation	PARP, TREX1	SLE, RA
Cytokine	IRF5, STAT4, TNPAIP3	SLE, SS, RA
Epigenetic control	MECP2, UBE2L3	SLE

SLE, systemic lupus erythematosus; RA, rheumatoid arthritis; SSc, scleroderma; SS, Sjögren's syndrome.

whereas DNMT3a and DNMT3b methylate unmethylated DNA. DNMT3L and DNMT2 display weak, if any, DNMT activity. CpG pairs have been conserved through evolution, within clusters referred to as CpG islands, which are concentrated in the promoter regions of coding genes and act as regulators of transcription. When demethylated, CpG pairs allow binding of transcription factors. The CpG methylation process is counterbalanced by DNA demethylation, which can be passive during the cell cycle or active as recently demonstrated.[7–9]

In addition to a direct repressive effect on transcription factor binding, methylated CpG pairs can recruit repressive partners including DNMTs and methyl-CpG-binding proteins (MBD). In turn, the repressive partners recruit supercomplexes such as the Mi2/NuRD (nucleosome remodeling and deacetylase). As a result, the nucleosome, a basic subunit of chromatin composed of 146 bp of DNA associated with an octamer of histones (H2A, H2B, H3 and H4), is subjected to post-translational modifications that affect chromatin accessibility. The main repressive modifications are related to histone acetylation under the control of histone deacetylase (HDAC) and histone methylation by histone methyl transferases such as suv39H1 and G9a. Histone post-translational modifications are not stable. Histone acetylation is reversed by histone acetyl transferases whereas histone methylation can be reversed by demethylating enzymes. Of note, in some cases, histone methylation is not repressive but active. In addition, although less characterized, phosphorylation, ubiquitination and deimination can also affect the nucleosome complex.[10]

DNA methylation

DNA methylation status in AID

The importance of DNA methylation in AID and especially in SLE was established in the 1960s and has been further strengthened since.[11] The pioneer ascertainment was related to the observation that two DNA demethylating drugs, procainamide and hydralazine, induce an SLE-like disease after long-term administration in normal mice. This effect disappeared when the drug was removed, and variations exist depending on the animal strain, sex and age. Of note, 5-azacytidine, another inhibitor of DNA methylation, delays the disease when added to the lupus-prone mice MRL/Lpr.[12] The implication of lymphocytes was provided by two groups, showing, respectively, after passive transfer of pretreated cells, the demethylated $CD4^+$ T cells and demethylated B cells in the autoreactivity process.[13,14] Translating this to humans, it was confirmed that DNA demethylation is defective in patients with AID.[15–18] Indeed, a lower content of DNA 5-methylcytosine is characteristic in peripheral blood mononuclear cells (PBMC) in patients with SLE, synovial mononuclear cells and synovial tissues in RA patients and cerebral cells in MS patients. The main point that emerges from the studies is the fact that cells with DNA hypomethylated are different from one AID to another. Thus, it explains why PBMC are not the gold standard to highlight epigenetic modifications as described in dermatomyositis, MS and primary biliary cirrhosis.[16,19,20]

Endogenous retroelements are deregulated in AID

Scattered throughout the genome, there are approximately 3 million endogenous retroelements. They are divided into long terminal repeats (LTR) including human endogenous retrovirus (HERV) and non-LTR elements, accounting for 8% and 34% of the genome, respectively. Endogenous retroelements have been considered for a long time as susceptible markers for autoimmunity as DNA demethylation increases their expression. This was demonstrated for non-LTR Alu retroelements that represent 55% of the cell-free DNA in SLE patients *v.* 13% in controls,[21] and for expression of HERV elements that are detected at a higher frequency in AID.[22] Additionally, allelic variants are associated with the development of AID: HTLV-1 related endogenous retroviral sequence (HRES-1) in SLE and HERV-K18 in MS and diabetes type 1.[23–25] As a consequence, the direct implication of endogenous retroelements in the development of AID may be suspected, and several observations support this hypothesis. First, Alu RNA retroelements can form auto-antigenic complexes with nascent histones or ribonucleoproteins.[26] Second, when transcribed, the HERV proteins, like the HRES-1 p30 gag protein, can be recognized by antibodies

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(Ab) and such Ab cross-react with the SLE auto-antigen U1-snRNP. The anti-HRES-1 p30 gag/U1-snRNP Ab are detected in up to 50% of SLE patients *v.* less than 5% in controls.[27] Another example is the HERV-W envelope protein that can act when overexpressed in MS as a super antigen, leading to the expansion of autoreactive T cells.[28] Third, when integrated into or adjacent to an immune-related gene, an endogenous retroelement could interfere with expression of the gene. One example is the HERV-CD5 endogenous retrovirus integrated upstream of the *cd5* gene 25–50 million years ago at the time of divergence between Old and New World monkeys.[29]

DNA methylation controls lymphocyte autoreactivity

Lymphocyte differentiation is a step-wise process that starts in the bone marrow from hematopoietic multipotent stem cells, and each step leads to specific epigenetic modifications.[30,31] The alteration of DNA methylation/demethylation is necessary for cytokine polarization in T helper cells, the selection of regulatory T cells and possibly B cell and the control of lymphocyte autoreactivity via rearrangement of the antigen receptor gene. Indeed, blocking DNA methylation with specific inhibitors in activated B- and T-cells leads to the emergence of autoreactive lymphocytes and development of an SLE-like disease with detection of anti-nuclear antibodies when demethylated lymphocytes are reinjected into mice.[13,14]

The implication of DNA methylation in the control of lymphocyte autoreactivity is reinforced by the analysis of lymphocytes in the immunodeficiency centrometric region instability and facial anomalies syndrome (ICF), which is characterized by a non-functional DNMT3b. In these patients, there is an absence of mature T cells and accumulation of immature B cells with an autoreactive B cell receptor.[32]

The DNA methylation process is impaired in AID

DNA methylases are downregulated

Currently, most studies on DNMTs have been performed on $CD4^+$ T cells, revealing that the activation of DNMT1 is impaired in patients with SLE, systemic sclerosis and dermatomyositis.[33] DNMT3a and DNMT3b variations are also reported but their contribution in lymphocytes seems minor when compared with DNMT1.[33,34] More recently, DNMT1 defects have been observed in B cells from SLE patients and synovial fibroblasts from RA patients.[17,34] In $CD4^+$ T cells from SLE patients, a defect in the PKC delta kinase has been proposed to lead to the downregulation of the Raf/MEK/ErK/DNMT1 pathway.[35] Not surprisingly, the PKC delta knock-out mice develop an SLE-like disease with B cell expansion and autoantibody production.[36] Of note, the DNMT defect is more pronounced when cells are stimulated by phytohemagglutinin for $CD4^+$ T cells and IgM for B cells, explaining the conflicting results when using unstimulated cells and/or PBMC. [37,38] Another source of discrepancies is related to the selection of controls since variations are observed between young and old and between females and males.[39]

DNA demethylases are upregulated

Whereas DNA methylation has been extensively studied in eukaryotes, the demethylation process is poorly understood. A two-step model has been proposed recently in zebrafish embryos[40] and confirmed in mice.[8,9] In the first step, an apolipoprotein in B editing catalytic polypeptide deaminase (Apobec), such as the activation-induced cytidine deaminase (AICDA), converts 5 methyl-cytosine to thymidine. In the second step, the MBD4 DNA glycosylase repairs T:G mismatches by converting the thymidine to an unmethylated cytidine. The apobec/MBD4 complex requires a third partner, the UV radiation stress sensor Gadd45-alpha, to be active.

In $CD4^+$ T cells from SLE patients, the levels of Gadd45-alpha and MBD4 are inversely proportional to the global DNA methylation content, suggesting that an active demethylation process is at work in these cells.[41,42] In B cells, AICDA expression is controlled by DNA demethylation,[43] and an AICDA knock-out protects lupus-prone mice from lupus nephritis, suggesting that DNA demethylation is also active in SLE B cells.[44]

The polyamine hypothesis

A sufficient level of the methyl group donor SAM is required for an effective DNA methylation process.[45] As a consequence, increased SAM degradation impacts intracellular DNA methylation. Based on the fact that SAM decarboxylase activity is positively regulated by putrescine, a polyamine precursor, a new theory linking SAM reduction, polyamine overexpression and autoimmunity has been proposed.[46] The theory is reinforced by the observation that blocking putrescine production inhibits disease manifestations in a lupus-prone mouse model.[47] Furthermore, increased polyamines and reduction of the SAM level have been demonstrated in sera from SLE patients[48] and in synovial fluids and urine from RA patients.[49,50] Such a hypothesis links environmental factors, such as the Epstein–Barr virus,[51] and interferon,[49] with the DNA demethylation process through the polyamine pathway. As a consequence, blocking the polyamine cycle and/or SAM decarboxylase may reveal the potential of epigenetic-based therapeutic treatment.[52]

Histone modifications in AID

DNA methylation and histone post-translational modifications are closely linked. As a consequence, it is not surprising to observe that DNA demethylation in SLE $CD4^+$ T cells is associated with histone acetylation and with histone H3 demethylation at position K9.[53] The observation that trichostatin A, an HDAC inhibitor, improves the disease when used in spontaneous autoimmune mouse models was surprising.[54] One explanation is that HDAC inhibitors target cells unaffected by DNA demethylation. Indeed, among the differences of DNMT

inhibitors that induce lymphocyte autoreactivity and autoAb production, trichostatin A blocks cytokine production by dendritic cells and upregulates regulatory T cells.[55,56] However, the rationale for the use of HDAC inhibitors in AID patients is still debated, since a risk exists that inhibition of HDAC would result in aberrant gene expression in those demethylated cells.

Micro-RNAs

miRNA and autoreactivity

miRNAs are genome-encoded 21- to 23-base pair (bp) RNAs that target the 3′ untranslated region (UTR) of specific messenger RNAs (mRNA) for degradation or translational repression. In humans, one thousand miRNAs are suspected to exist, each of them having the capacity to target up to 100 transcriptional genes, suggesting that much of the transcriptome is regulated by miRNAs. miRNAs play a pivotal role in both innate and adaptative immunity, and in immune cells by controlling their development, maturation and functions. In mice expressing high levels of miR-17-92 in lymphocytes, it was observed that miRNA dysregulation leads to AID.[57] These mice develop lymphoproliferative disorders associated with an SLE-like disease that includes nephritis with anti-dsDNA deposition. In all, two miR-17-92 targets have been characterized: the pro-apoptotic protein Bim and the tumor suppressor PTEN.

miRNA and DNA methylation

Like regular genes, miRNAs could be regulated by DNA methylation, but the opposite is true since miRNAs can target DNMT1 (miR-21, miR-126, miR-148a, miR-152), HDAC1 (miR-449a) and MBD2 (miR-373), leading to an amplification loop (Table 2).[58–61] It can also be speculated that each of these miRNAs are counterbalanced by other miRNAs.

Altered miRNA expression in AID

miRNA dysregulation has been reported in several AID including SLE, RA, pSS, diabetes and MS.[5] In addition to the identification of miRNAs associated with AID, functional analysis has highlighted the contribution of miRNA as an overlap mechanism in AID. First, miR-155, known to be implicated in cellular proliferation and cytokine production, is commonly detected in defective regulatory T cells from SLE patients,[62] RA peripheral blood and fibroblast-like synoviocytes from RA patients,[63] and in salivary glands isolated from pSS patients [Y.R. and P.Y. unpublished data]. Second, miR-146 is differentially regulated between SLE and RA. In the latter, miR-146 overexpression controls the IFN pathway but not in the former.[64] Third, three miRNAs (miR-21, miR-126 and miR-148a) contribute to DNA demethylation in $CD4^+$ T cells by targeting DNMT1 directly or indirectly.[38–59] This provides another explanation for the DNA demethylation process observed in $CD4^+$ SLE T cells.

Table 2. *Micro-RNAs control chromatin accessibility*

miRNA	Target gene	Associated diseases
miR-21	DNMT1	SLE[58]
miR-126	DNMT1	SLE[59]
miR-148a	DNMT1	SLE[58]
miR-373	MBD2	Cholangiocarcinoma[61]
miR-449a	HDAC1	Prostate cancer[60]

SLE, systemic lupus erythematosus.

However, these miRNAs can be upregulated when using inhibitors of DNA methylation, thus suggesting that they are not a primary event but contribute to amplification of the process.[65]

Post-translational modifications and AID

Citrullinization and antigen presentation

Pepdityl arginine demininase (PADI) enzymes catalyze the conversion of arginine residues in proteins into citrulline residues. Among the five human PADI, PADI4 is the only one able to move to the nucleus upon cellular activation. In the nucleus, PADI4 can operate on arginine or methylated arginine residues in N terminal regions of histones H2A, H3 and H4. As a consequence, transcription is repressed by preventing active histone modifications.[66] Furthermore, regarding its influence on transcription, PADI4 overexpression in RA contributes to the production of autoAb against citrullinated peptides. Many proteins have been described as targets of antibodies to citrullinated Ab (ACPA) that bind fibrin, vimentine, collagen and alpha-enolase. The recent description of PADI in the peridontal pathogen *porphyromonas gingivalis* provides a link between an environmental factor and the development of RA.[67] In MS, the PADI2 promoter is demethylated, thus causing overexpression and, as a consequence, the citrullinization of myelin basic protein (MBP) increases, resulting in a loss of myelin stability.[68]

Histone modification and autoantibodies

Histone post-translational modifications are important to control transcription, replication, cellular division, activation, necrosis and apoptosis. The latter is suspected to play a central role in numerous AID, and consequently, it is not surprising to observe that, related to cell death, histone post-translational modifications are associated with autoimmunity, such as H3K27me3, H2BK12ac, H3ac and H4ac.[10] The importance of this process in AID is reinforced by the observation that administration of a very low dose of H4 peptides containing autoepitopes in lupus-prone mice reduces autoantibody production, extends the lifespan and protects the kidneys from glomerulonephritis.[69]

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CD5 B cell model

SLE B cells are characterized by a high production of IL-6, a DNA demethylation profile, and a reduction of the B cell receptor (BCR) with dampened CD5 at the cell surface.[34] Observing that CD5 expression was regulated by DNA methylation inhibitors and by blocking the IL-6 autocrine loop on SLE B cells, we have suspected and demonstrated that IL-6, by controlling DNA methylation, regulates CD5 cell surface expression. First, it was demonstrated that IL-6 controls DNMT1 expression and DNA methylation at the CD5 locus by blocking the cell cycle. Second, DNA demethylation at the CD5 locus is associated with overexpression of an alternative transcript, called CD5-E1B, which results from the fusion of an HERV element with exon 2 of CD5. Finally, when expressed, CD5-E1B codes for a truncated variant that interacts with the classic form of CD5 and downregulates its cell surface expression.[70,71] Following this line of reasoning, blocking the autocrine IL-6 loop restores the DNA demethylation in SLE B cells and prevents autoantibody production, thus providing a new therapeutic strategy.

Conclusion

In contrast to the importance of epigenetic studies performed in cancer, and in cardiovascular diseases, only a small number of laboratories have studied epigenetic mechanisms in the context of AID. As a consequence, epigenetics in AID is at its infancy. However, the results appear to confirm the role played by the epigenetic process in AID development. Undoubtedly, the development of novel epigenetic technologies, the characterization of epigenetically dysregulated genes and the development of new therapeutic strategies based on epigenetic control will provide new avenues in AID.

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Statement of interest

None

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