REVIEW

The epigenetics of autoimmunity

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The etiology of autoimmune diseases remains largely unknown. Concordance rates in monozygotic twins are lower than 50% while genome-wide association studies propose numerous significant associations representing only a minority of patients. These lines of evidence strongly support other complementary mechanisms involved in the regulation of genes expression ultimately causing overt autoimmunity. Alterations in the post-translational modification of histones and DNA methylation are the two major epigenetic mechanisms that may potentially cause a breakdown of immune tolerance and the perpetuation of autoimmune diseases. In recent years, several studies both in clinical settings and experimental models proposed that the epigenome may hold the key to a better understanding of autoimmunity initiation and perpetuation. More specifically, data support the impact of epigenetic changes in systemic lupus erythematosus, rheumatoid arthritis, multiple sclerosis and other autoimmune diseases, in some cases based on mechanistical observations. We herein discuss what we currently know and what we expect will come in the next future. Ultimately, epigenetic treatments already being used in oncology may soon prove beneficial also in autoimmune diseases.
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WHY EPIGENETICS?

Autoimmune diseases are generally considered as complex (and/or multifactorial) diseases. Genetic background confers susceptibility to or protection from disease onset, but it is neither sufficient nor causative for disease development. While numerous similarities between conditions are being identified,[1] the etiology of the majority of autoimmune diseases remains largely unknown. Although strong genetic bases have been found by recent genome-wide association studies,[2] these studies fail to demonstrate the presence of a unique genetic mechanism underlying immune tolerance breakdown and, moreover, the significant genetic associations identified are found only in relative small proportion of patients.

The largely incomplete concordance rates of autoimmune diseases in monozygotic (MZ) twins (Table 1) strongly support other complementary mechanisms involved in gene expression regulation ultimately causing overt autoimmunity. Based on these observations, the use of novel strategies focusing on the analysis of histone modifications and DNA methylation supports the notion that epigenetic alterations may play a crucial role in triggering autoimmunity. Epigenetics (from the Greek $\varepsilon\pi$ (epi) over and $\gamma\varepsilon\nu\varepsilon\tau\iota\kappa\varsigma$ (genetics)) studies mechanisms that determine and/or perpetuate heritable genomic functions without changes in DNA sequence.

Epigenome and/or epigenotype is, thus, considered as a cell-specific and stable pattern of gene expression induced by such epigenetic mechanisms. Functionally, epigenetic mechanisms are, indeed, crucial for cell type development and differentiation, being able to induce stable expression or repression of genes. Epigenetic mechanisms are, also, able to confer a metabolic plasticity to cell, thus allowing the cell to adapt itself to environmental changes.

The potential role of epigenetics in environmental/genetic interactions, where environmental changes produce modifications in gene expression, has been suggested by some intriguing experimental studies.

Firstly, a seminal study investigated the use of a specific dietary regimen, i.e. foods rich in methyl donors, in order to modify coat color in *agouti* pregnant rodents. Such regimen led the offspring to manifest a specific coat color compared to mothers fed with a standard diet. This observation has been explained by an altered DNA methylation process that is the most thoroughly studied epigenetic mechanism. Such process silences the intracisternal A particle retroviral insertional element, ultimately limiting the appearance of *agouti* alleles. A second major example came from Dutch individuals who were exposed to famine during intrauterine life and childhood during the World War II. The DNA methylation analysis of the region regulating the insulin-like growth factor 2 (IGF2) expression constitutes a major example of epigenetic imprinting by demonstrating subjects a well-conserved hypomethylation status in exposed compared to non-exposed subjects.[3]

Recent observational studies have shown association of DNA methylation profiles with several environmental factors including exposure to prenatal tobacco smoke,[4] alcohol consumption,[5] and environmental pollutants.[6,7] Based on these observations, it is becoming clear how epigenetic mechanisms should be considered as the new frontier in the interaction between genome and environment, thus well conjugating the *adagio* stating that complex diseases ensue from 'bad genes and bad luck'. This was strongly supported by the experimental data proposed by Dr Fraga and colleagues who demonstrated how epigenetics may well explain the discordance of autoimmune

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Table 1 Pairwise CRs of autoimmune diseases in MZ and DZ twin sets were calculated as *n* of concordant sets/*n* of studied sets

	MZ twins CR	DZ twins CR
Systemic lupus erithematosus	0.24	0.02
Sjögren's syndrome	Concordant pair reported	—
Type I diabetes mellitus	0.21–0.70[a]	0.00–0.13
Rheumatoid arthritis	12.3–15.4	3.50–3.60
Primary biliary cirrhosis	0.63	0.00
Primary sclerosing cholangitis	Concordant pair reported	—
Graves' disease	0.17–0.29	0.00–0.02
Multiple sclerosis	0.25–0.31[a]	0.03–4.7
Celiac disease	0.75–0.83	0.11

Abbreviations: CR, concordance rate; DZ, dizygotic; MZ, monozygotic.
[a] 7.5 years of observation.

diseases in MZ twins.[3] Phenotypic differences significantly increased along with age of the twins in a trend coined as 'epigenetic drift', which occurs during life according with the different expositions to environmental stressors.[8]

The present article will first illustrate the major epigenetic mechanisms under investigation and then will discuss available data in the field of autoimmune diseases.

EPIGENETIC MECHANISMS

All epigenetic mechanisms share the feature of not altering DNA sequence, but only the possibility that gene sequences are transcribed under specific conditions. Comparing prokaryotic and eukaryotic organisms may help our understanding of how epigenetic mechanisms can regulate gene expression. Prokaryotes do not have nucleus and have a limited number of genes, whose transcription is regulated by factors binding directly to DNA promoter elements.

In contrast, eukaryotes have a significantly larger number of genes that have to be compacted in order to fit within the cell nucleus. In order to do so, genes are packed together with specific proteins, i.e. histones, in an ultra-structure known as chromatin. This DNA functional packaging system has regulatory functions, as chromatin can undergo steric changes, thus being able to interfere with proteins involved in gene transcription. Similarly, transcription regulation is derived from the methylation of cytosine DNA residues (particularly within gene promoters) which inhibits the transcription of the downstream genetic sequence. DNA methylation and histone modifications constitute the major epigenetic changes known so far, and therefore their underlying processes will be discussed below in further details. Moreover, the newest field of microRNA (miRNA) will be briefly illustrated as an additional gene regulatory mechanism.

Histone modifications

As mentioned before, histones are highly conserved proteins that reside within nuclei of eukaryotic cells. They can be classified into two main groups: (i) core histones (H2A, H2B, H3 and H4) that are part of the nucleosome core, the basic unit of DNA packaging in eukaryotics; and (ii) linker histones (H1 and H5). Two of each of the core histones assemble to form an octameric nucleosome core particle by wrapping about 147 base pairs of DNA around the protein spool in a 1.7 left-handed super-helical turn[9] (Figure 1), thus providing DNA condensation and organization in the nucleus, as well as modulating DNA accessibility to the transcription machinery. This latter process could be represented as a drawer that can be opened or closed following specific stimuli. In fact, each histone subtype can
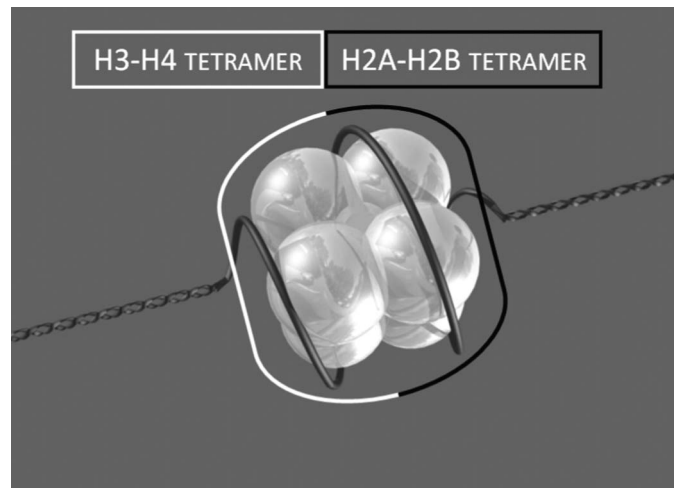

Figure 1 The structure of the nucleosome. The figure depicts the histone composed of two tetramers with DNA wrapped around the proteins.

be modified by different chemical modification at defined amino acids leading to transcription modulation and, therefore, cell cycle regulation, development and differentiation.

Each of the four core histones shares the same folding structure known as histone fold domain, which consists of three α-helices (α1, α2 and α3) separated by two loops (L1 and L2).[10] The histone fold domains fold together in antiparallel pairs (H3 with H4 and H2A with H2B) to constitute tetramers. The subsequent assembly of two tetramers forms the octameric core structure (H3/H4-H2A/H2B1) of the nucleosome.[11] The N-terminal regions of histones protrude outside the nucleosome core and are prone to post-translational modifications, which are important in chromatin compaction and gene regulation. Histone post-translational modifications concur to determine the pattern defined as 'histone code' and will be summarized below. All these histone modifications are caused by specific enzymes which recognize histone tails and can work to add or remove functional groups which are in turn recognized by nuclear factors. Specific proteins have affinity for modified amino acid residues (for instance bromodomains bind acetylated lysines or chromodomains methylated lysines) and promote specific changes in chromatin determining respectively the activation or the silencing of gene transcription (Figure 2).

Among histone modifications, acetylation and deacetylation are one of the most important gene expression regulatory mechanisms. These processes involve selected lysine residues in the tails of nucleosomal histones and are induced by histone acetyltransferase (HAT) and histone deacetylase (HDAC) enzymes, respectively.[12–15] HAT enzymes share the ability to promote gene expression by transferring acetyl groups to lysine[16–18] while HDACs remove acetyl groups and generally associate with gene repression.[19–21] A second mechanism involves histone methylation and its effects depend on the position of the modified lysine residue within the histone tail and on the number of methyl groups added to such residues. As an example, the presence of three methyl groups on lysine 4 residue on histone H3 (Me-H3K4), has been associated with transcriptional activation whereas the triple methylation of residues 9 or 27 determines repression.[3,22–26]

As a third mechanism, arginine can also be methylated/demethylated by specific enzymes and play a critical role in the dynamic regulation of gene expression.[27] Methylation of arginine residue 3 on histone H4 (H4R3) and arginine 17 on histone H3 (H3R17) have been shown to induce gene activation.[23,28–30] Finally, ubiquitin is a 76
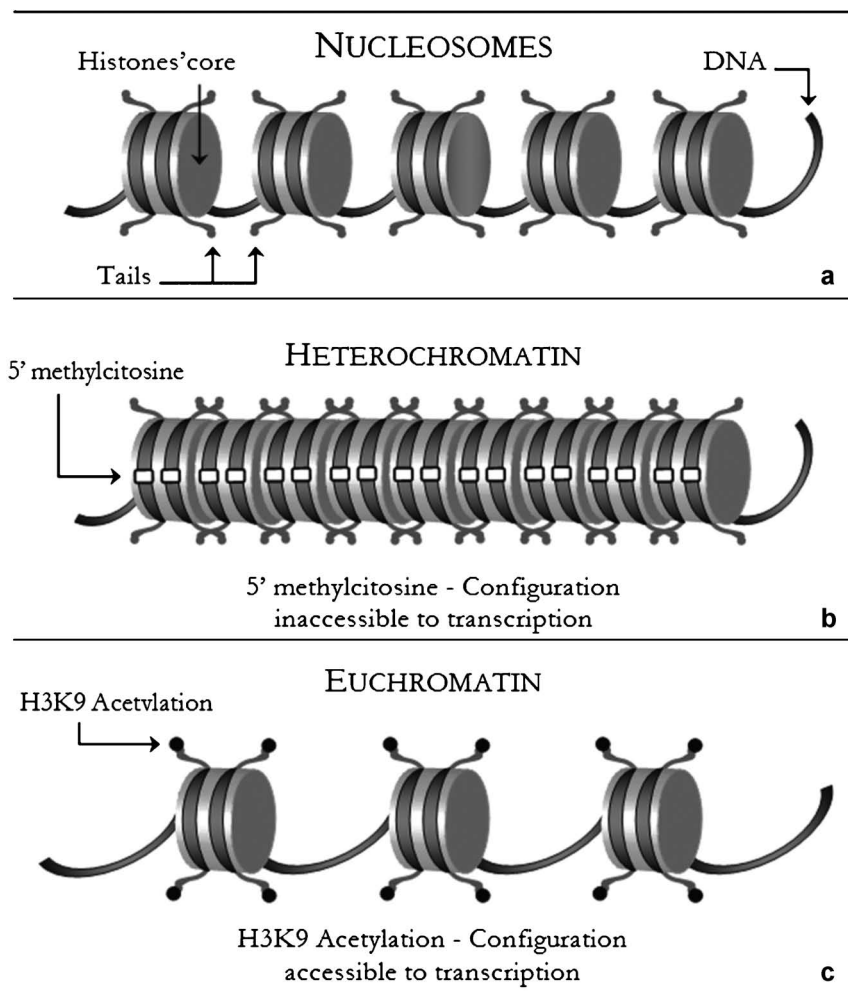
NUCLEOSOMES

Histones'core

DNA

Tails

a

HETEROCHROMATIN

5' methylcitosine

5' methylcitosine - Configuration
inaccessible to transcription

b

EUCHROMATIN

H3K9 Acetvlation

H3K9 Acetylation - Configuration
accessible to transcription

c

Figure 2 (**a**) Nucleosomes: the interactions between eight histone proteins determine a quaternary structure which leads to a double wrapping of DNA molecule. (**b**) Heterochromatin: histone deacetylation with the association of other histone modifications confers a dense configuration to DNA molecules. (**c**) Euchromatin: the epigenetic process of histones' tails acetylation is usually associated with an active configuration.

amino acid protein that is involved in specific protein labeling. Ubiquitinated proteins are committed to proteosomal degradation and ubiquitination thus controlling the stability and intracellular localization of numerous proteins. Ubiquitination ultimately influences the status of histones methylation or acetylation[31] to modulate gene expression, as in the case of the nuclear factor-κB pathway.[32]

DNA methylation

DNA methylation consists of the addition of a methyl group to the fifth carbon of cytosine residues, converting these to 5-methylcytosines. This reaction involves specific enzymes called DNA methyltransferases (DNMTs) and a methyl group donor, S-adenosylmethionine (Figure 3).

Among DNMTs, DNMT3A and DNMT3B are responsible for *de novo* methylation, while DNMT1 maintains epigenetic covalent modifications during cell replication. In mammalian genome, DNA methylation occurs mostly at CpG islands that are regions exceeding 500 base pairs with a CG content higher than 55%.[33,34] CpG islands have key regulatory functions, and can be found in promoter regions of about half of all genes.[35] Altered CpG island methylation may, indeed, change chromatin structure, being typically able to modulate the finely promoter–transcription factor interactions within the transcription machinery.[36]

As a consequence, in most cases, both acquisition and somatic maintenance of such methylation state induce gene repression. Moreover, CpG-methylated sites can also interact with specific proteins containing a domain called methyl-CpG-binding domain. Although their specific contribution to different sites in different cell contexts is still unclear, methyl-CpG-binding domain proteins have been proposed to induce gene repression by binding to methylated DNA, a process that would lead to chromatin modification and re-modeling complexes.

The importance of DNA methylation process in gene expression is well represented by sex chromosome balance, where females are characterized by partial transcriptional suppression of X chromosome obtained through methylation of specific genetic sequences.[37] Mammalian genome contains dispersed clusters of genes, whose specific allele expression is determined by inheritance of parental methylation status. These genome regions that are rich in CpG and by which an organism inherits a state of expressed or unexpressed genomic sequence from parents, are usually referred as imprinting centers. Therefore, errors in methylation of such imprinting centers can induce an inherited altered gene expression.

From a clinical standpoint, the importance of DNA methylation, and in particular, of an impaired methylation, is suggested by the two
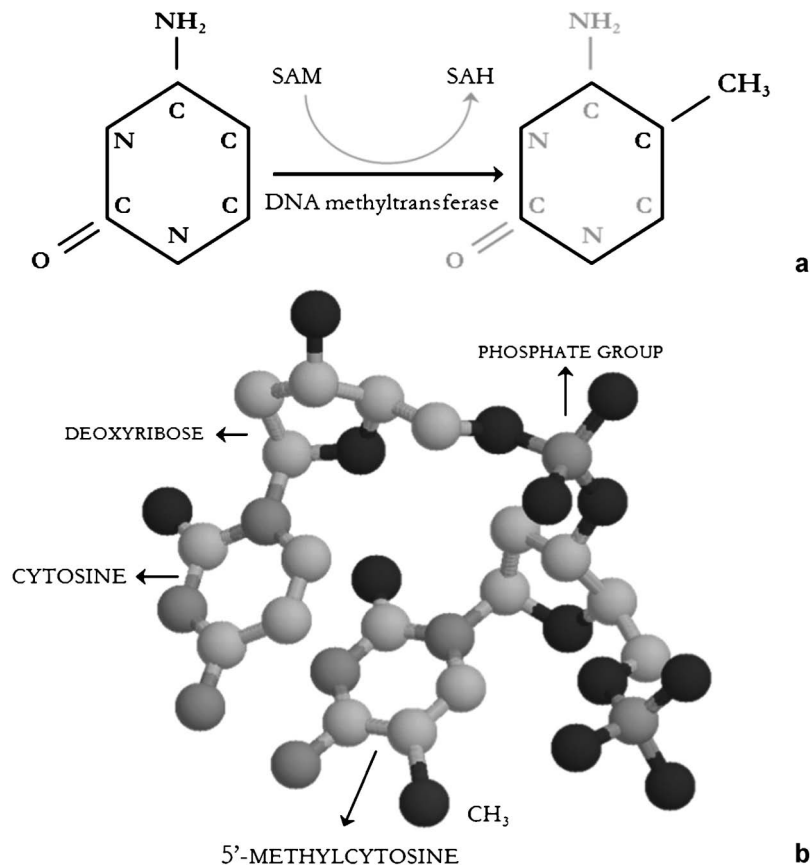
Figure 3 Panel a: Biochemistry of DNA methylation. DNA methyltransferase catalyze the reaction. The enzyme shifts the methyl group from SAM to the fifth carbon of cytosine. The reaction produces 5′-methylcytosine and SAH. Cytosine bases are integrant part of DNA filament and cannot be found as free molecules. Panel b: Cytosine and 5′-methylcytosine molecular structure in a DNA fragment. The brown atom represents the methyl group -CH$_3$. Grey: carbons; blue: azotes; red: oxygens; yellow: phosphates. SAH, *S*-adenosylhomocysteine; SAM, *S*-adenosylmethionine.

rare congenital diseases known as Silver–Russel and Beckwith–Wiedemann syndromes. Both conditions derive from errors in methylation of a specific imprinting center located on chromosome 11 between genes encoding IGF2 and imprinted maternally expressed transcript (H19). In physiological condition this center is methylated only on the paternal allele. Beckwith–Wiedemann syndrome is characterized by an overexpression of IGF2 due to methylation of both parental alleles, while, in a complementary fashion, the double loss of imprinting centre methylation suppresses IGF2 locus and increases H19 expression causing Silver–Russel syndrome.

miRNAs

miRNAs are a group of post-transcriptional regulators involved in many biological processes including development, differentiation, proliferation and apoptosis.[38] miRNAs are about 22 nucleotide-long non-coding RNAs that suppress translation by binding to complementary target mRNA species and causing the degradation of the target.[39] miRNAs are genome encoded and transcribed by RNA polymerase II, similar to ordinary protein-coding RNAs[40] and were recently investigated in autoimmune and chronic inflammatory conditions.[41]

One of the best known examples of epigenetic regulation due to miRNA–DNA interactions is represented by X-chromosome inactivation in women. Indeed, one of the two X chromosomes in female organisms is silenced by epigenetic mechanisms leading to dosage compensation of X-chromosome products. This balance is obtained

with the transcription of X-inactive-specific transcript gene from the so called X-inactivation center and it leads to adapt gene expression in female sex with male organisms.[42] X-inactive-specific transcript codes for a non-coding mRNA that coats one X chromosome, so this process silences the expression of a great part of genetic sequences on the future inactivated X chromosome.[43] Silencing is stabilized by the addiction of repressive histone marks and DNA methylation. For these reasons it has been assumed that errors in epigenetic X-chromosome silencing could be involved in the pathogenesis of several diseases, including autoimmune diseases. This is indeed an attractive hypothesis that could explain the noticeable female predominance in autoimmune related disorders.[44]

AUTOIMMUNE DISEASES AND EPIGENETIC MODIFICATIONS

The impact of epigenetics is rapidly increasing in all complex diseases and is soon expected to gain a more prominent role in our future understanding of medicine. In general terms, we may well hypothesize that epigenetics will fill the gap between genomics and environmental factors in the pathogenesis of this type of conditions.

In the case of autoimmunity, presence of specific impairments in the regulation of epigenetic processes in immune cells would be responsible for immune-tolerance breakdown through both hypomethylation of genes or involvement of transcription repressors.[45–47]

In autoimmune diseases, epigenetics is mostly studied using peripheral blood mononuclear cells in humans or using animal models. Both *in vitro* and *in vivo* experimental models have shown that

variation of the epigenome may lead to the onset of autoreactive T-cell clones. Specific epigenetic defects have been associated with auto-immune disorders.

As an example, the differentiation of T helper cells to Th1 subsets producing interferon-γ (IFN-γ) and acting against bacteria or Th2 subpopulations producing interleukin-4 (IL-4) and IL-13 cytokines is epigenetically regulated. Th1 cells present an IFN-γ demethylated promoter but have repressive epigenetic histone modifications at IL-4-13 locus, opposite to what is observed in the Th2 subsets.[48] This is an example of the interactions between immune system dysregulation and epigenetics but the loss of immune tolerance is a complex process difficult to investigate particularly for non-traditional antigens.[49] The study of specific models may help to understand the mechanisms involved in tolerance breakdown; one major example is represented by a unique human model: MZ twins.[50]

MZ twins are an ideal model to study environmental and epigenetic influences which could contribute to the autoimmune process, as proposed by Francis Galton, an English scientist of the nineteenth century. It was Galton's work published in 1874 in his book 'English man of science: their nature and nurture' that constitute the basis for current epigenetics.[3,51] Over a century later, Fraga et al.[8,52] demonstrated the presence of an epigenetic drift in several MZ twin pairs and highlighted the importance of epigenetic modifications in the development of differences between twins. From a clinical standpoint, the concordance rates for autoimmune diseases in MZ twins are a powerful tool to determine the impact of genetics and the environment in determining disease onset. Concordance rates among MZ twins vary widely but, with only two exceptions, are well below 50% (Table 1), thus making this model an ideal target to investigate the role of epigenetics. Recent years have witnessed an increasing number of studies investigating epigenetics in specific autoimmune diseases and will be discussed in the sections below and are summarized in Table 2.

Systemic lupus erithematosous (SLE)

SLE is a systemic multiorgan autoimmune disease with different immunological and clinical manifestations characterized by an autoantibody response to nuclear and/or cytoplasmic antigens.

Most recent genome-wide association studies demonstrated that genomics significantly predispose to SLE onset,[53–57] but the incomplete disease concordance between identical twins suggests a role for other complementary factors.[52,58] In this undefined scenario, experimental data indicate that epigenetic mechanisms, and in particular impaired T- and B-cell DNA methylation, may constitute one of these factors.[59]

Several studies have uncovered the importance of DNA hypomethylation in SLE etiology,[60,61] and in particular it has been suggested that this phenomenon may affect the structure of T-cell chromatin, resulting in cellular hyperactivity. Changes in DNA methylation are regulated by the extracellular signal-regulated kinase signaling pathway[62] and this pathway is reduced in murine T cells causing a decreased expression of DNMT1 and an overexpression of methylation-sensitive autoimmunity genes, similar to T cells in human SLE.[63] Human data further confirmed these views as T cells from patients with active SLE manifest decreased total deoxymethylcytosine content and decreased DNMT1 transcripts[64] leading to the hyperexpression of several genes. Epigenetic similarities between patient lymphocyte and experimentally demethylated T cells were also demonstrated with SLE cells capable of stimulating antibody production by autologous B cells.[65,66] Another line of evidence came from the elevated CD70

Table 2 Available evidence on the epigenetics involvement in specific autoimmune diseases

Systemic lupus erithematosus	T- and B-cell global DNA hypomethylation[60,61] with decreased DNMT1 transcription[64]
	miR-146a probably involved in disease onset[82]
	CD4+ T-cell changes:
	• CD70 demethylation;[65,67]
	• CD40L demethylation (in women);[68]
	• hypoacetylation of histone proteins H3 and H4;[81]
	• H3 acetylation negatively correlates with disease activity.[81]
	B-cell changes:
	• CD70 demethylation;[67,79]
	• perforin demethylation.[67,79]
Rheumatoid arthritis	RASF changes:
	• global DNA hypomethylation;[92]
	• hypomethylation of CpG islands in LINE-1 promoter;[92,93]
	• hypomethylation of DR-3 promoter;[95]
	• unmethylated CpG in IL-6 promoter;[94]
	• miR-155,[100] and miR-146 (Ref. 102) upregulated.
Systemic sclerosis	Methylation of CpG islands in FL1 promoter with reduced expression[107,109,111]
Sjögren's syndrome	Upregulated miR-574-3p and -768-3p in salivary glands[114]
	Upregulated miR-150 and -146 in salivary glands and lymphocytes[115]
Type 1 diabetes mellitus	Global hypermethylation activity caused by altered metabolism:
	• glucose and insulin levels increase methylation by altering homocysteine metabolism;[130,179–181]
	• low protein diet decreases islet mass and vascularity.[134,135]
Multiple sclerosis	PAD2 hypomethylation in white matter cells[150,151]
	Effects of trichostatin A (histone deacetylase inhibitor) in murine models[157]

Abbreviations: DR-3, death receptor 3; DNMT, DNA methyltransferase; RASF, rheumatoid arthritis synovial fibroblast; PAD2, peptidyl arginine deiminase, type II

expression in SLE cells similar to what is observed *in vitro* stimulating $CD4^+$ T cells with the epigenetically active molecules procainamide and hydralazine, as both drugs cause CD70 demethylation.[65,67] Further, cells from women with SLE overexpress CD40L and manifest a demethylation of the corresponding gene on the inactivated X chromosome. Since cells from male patients do not overexpress CD40L,[68] this finding has been advocated to explain SLE female predominance. The common trait of these observations could reside in the epigenetically-mediated downregulation of the transcription factor RFX1 in $CD4^+$ T cells.[69]

The study of 5-azacytidine to understand SLE pathogenesis was prompted by the clinical evidence of a lupus-like syndrome in patients treated with procainamide and hydralazine. It has been found that $CD4^+$ T cells treated with an inhibitor of DNMT1, such as 5-azacitidyne, become autoreactive, and the process is reversible after the drug is discontinued.[61] The study of these patients demonstrated that only a group of treated subjects develop the syndrome, thus suggesting the presence of an idiosyncratic reaction which remains one of the most studied phenomena in modern pharmacology. These rare adverse reactions arise in a restricted subset of people. This group of patients is difficult to treat. Moreover, it is impossible to predict what element of the human population could develop the reaction. The most important drugs involved in induction of a lupus-like disease are procainamide and hydralazine, even though both cause antinuclear antibodies, ANA in a majority of people.[70] The development of a systemic involvement and clinical manifestation probably require the presence of lupus susceptibility genes.[71] Interestingly, both drugs are DNA methylation inhibitors but procainamide is a competitive inhibitor of DNMT1 enzymatic activity[72] and hydralazine inhibits T- and B-cell signal-regulated kinase pathways.[73,74] The observation of an increased expression of adhesion molecules on lupus drug-induced lymphocytes proves the epigenetic mechanisms and their role in the induction of autoreactivity.[75] Similarly, lupus $CD4^+$ T cells have an abnormal interaction with major histocompatibility complex (MHC) molecules as supported by the experimental evidence of an abnormal self-antigen response following treatment with 5-azacytidine[76] which demethylates sequences encoding costimulatory molecules like CD11a.[77,78] These molecules take part in $CD4^+$ T-cell activation and their hyperexpression influences lymphocytes interaction with self-antigens. Furthermore, 5-azacytidine demethylates the cytotoxic molecule perforin and the B-cell costimulatory molecule CD70 causing their overexpression, both phenomena being observed also in patients' T cells.[67,79] The increased perforin and CD70 expression levels contribute to autoreactive macrophage killing capability which can generate a source of antigenic apoptotic nucleosomes,[79] and antibody overproduction,[65] respectively. One recent study compared DNA methylation in genome-wide loci in a cohort of MZ twins discordant for SLE, rheumatoid arthritis (RA) and dermatomyositis.[80] MZ twins discordant for SLE manifested DNA methylation and expression changes in genes relevant to SLE pathogenesis and a global decrease in the methylation content.

Histone modifications have been studied in both lupus mouse models and human lupus. Global acetylation of histones H3 and H4 in active SLE $CD4^+$ T cells was found to be decreased and H3 acetylation negatively correlated with disease activity.[81] A recent study also demonstrated that a negative regulator of the IFN pathway, miR-146a, may contribute to disease onset.[82] Both H3 and H4 histones are hypoacetylated in spleen-isolated cells from lupus-prone mice compared with controls.[83] As observed in DNA methylation, the use of the HDAC inhibitors such as trichostatin A or suberoylanilide hydroxamic acid

demonstrated improvement in glomerulonephritis and splenomegaly commonly observed in SLE.[84,85] These results are further supported by the *in vitro* use of HDAC inhibitors leading to increased histones H3 and H4 acetylation and reduced production of IL-12, IFN-γ, IL-6 and IL-10.[84] A murine strain carrying a HAT mutation develops a severe lupus-like disease with serum anti-dsDNA autoantibodies, glomerulonephritis and premature death.[86]

RA

RA is a chronic systemic inflammatory disease that primarily affects peripheral joints. As observed for SLE, the clinical onset RA requires a combination of genetic susceptibility factors, deregulated immunomodulation and environmental influences.[87–89] We may, then, hypothesize that only a genetic predisposition in concert with specific epigenetic alterations leads to the RA-associated immune system dysregulation. The typical joint localization of RA can be explained with the presence of local and environmental factors which remain unknown but may well include epigenetic changes.

In recent years, the epigenetics of RA have been widely investigated.[90] It has been proposed that RA synovial fibroblasts (RASFs) have a major role in the initiation and perpetuation of RA,[91] possibly *via* decreased global DNA methylation[92] or hypomethylation of CpG islands in LINE-1 promoter.[92,93]

Unmethylated CpG islands within IL-6 promoter gene in monocytes have been associated with a local hyperactivation of the inflammation circuit.[94] RA monocyte cells also manifest a change in methylation status of CpG islands within the promoter of death receptor 3 (DR-3), which is, then, downregulated inducing resistance to apoptosis.[95]

RA synovial tissues are characterized by a drift of the balance between HAT and HDAC activity toward the former[96] as supported by the proposed benefits induced by HDAC inhibitors[97] such as FK228 which inhibits joint swelling, synovial inflammation and joint destruction in murine RA models.[98] Furthermore, FK228 suppresses the production of vascular endothelial growth factor *in vivo* and blocks angiogenesis in synovial tissue in collagen antibody-induced arthritis.[99] Conversely, a twofold lower HDAC activity was reported in synovial extracts from RA patients compared to osteoarthritis patients.[96]

Lastly, it has also been suggested that specific expression and function of miRNA, in particular miR-155 and miR-146, might be involved in RA pathogenesis[100] as these are highly expressed in RASFs but not in osteoarthritis synovial fibroblast.[100,101] Tumor necrosis factor-α and IL-1β enhance miR-155 expression, which manifests a repressive effect on metalloproteinases expression in RASFs.[100] miR-146 is upregulated by proinflammatory molecules in RA synovial tissues and has a negative regulatory function of the nuclear factor-κB pathway of RA patients' monocytes.[102]

Systemic sclerosis (SSc)

SSc or scleroderma is a rare condition of unknown etiology characterized by excessive collagen deposition in skin and other tissues with progressive vasculopathy. The presence of autoantibodies against nuclear autoantigens in patients with SSc, female predominance and frequent autoimmune comorbidity are considered signs of autoimmunity.[103,104] Aberrant fibroblast activation and collagen deposition ultimately lead to fibrosis with a gradual but progressive alteration of involved tissues and organs, a process characterized by an inbalance of stimuli favouring pro-collagen and a defective production of metalloproteinases.[105,106] This is particularly evident for the skin where the

increased collagen pool confers anelasticity to the derma causing the pathognomonic hard and thick appearance.

Cultured SSc fibroblasts manifest typical cellular abnormalities for multiple generations and maintain the profibrotic phenotype when transferred outside the disease environment,[107] thus suggesting the presence of an imprinted profibrotic cell phenotype. This phenotype is determined by an increase in production of a defined cytokines pool including TGF-β and other growth factors in association with reduced synthesis of matrix metalloproteinases 1 and 3. The clonal selection of profibrotic fibroblasts[108] is one of the proposed pathogenetic mechanisms but there are insufficient data to confirm this hypothesis and the causative mechanism could be represented by epigenetics. This hypothesis is primarily supported by Wang and colleagues, who reported an epigenetic influence on collagen gene expression by the addiction of DNMT and HDACs inhibitors in cultured SSc fibroblasts. Most recent studies identified a reduced expression of FL1,[107] a transcription factor that inhibits collagen production[109] with an inverse correlation between FL1 expression and type I collagen production in cultured fibroblasts.[110] An epigenetic regulation of FL1 is indirectly suggested by the presence of CpG islands in FL1 promoter that can be methylated and bound to specific regulatory proteins.[111] Epigenetic FL1 changes lead to increased collagen synthesis which is not balanced by metalloproteinase activity ultimately leading to collagen accumulation and fibrosis. This finding raises the possibility that aberrant DNA methylation within fibroblasts may contribute to the development of the disorder,[107] but genome-wide studies on DNA methylation and possibly histone modifications are awaited.

Sjögren's syndrome (SjS)

SjS affects salivary and lacrimal glands, resulting in dry mouth and/or dry eye conditions in patients as a consequence of autoimmune responses to self-antigens.[112] Despite extensive investigations into the etiology of SjS focusing on genetic, environmental and immune factors, neither the triggering nor the disease-initiating events have been identified.[113] Nevertheless, most recent data reported the overexpression of two miRNAs (namely, miR-574-3p and -768-3p) in the salivary glands of SjS patients,[114] while the study of non-obese diabetic mice with associated SjS demonstrated the upregulation of other miRNAs (miR-150 and -146) in target tissues and in peripheral lymphocytes.[115] The overexpression of miR-146 was confirmed in the salivary glands and peripheral lymphocytes of patients with SjS.[115]

Type 1 diabetes (T1D)

T1D is a T cell-mediated autoimmune disease[116] that develops in genetically susceptible individuals. In fact, predisposing genetic polymorphisms have been identified in T1D patients such as those in MHC class II (DR and DQ), insulin, PTPN22, CTLA4 and IL-rRA.[117] The disease incidence has been increasing over the past decades, as well represented by the data from Finland, where T1D yearly incidence has increased from 12 to 63 per 100 000,[118,119] somehow in conflict with MHC data.[120,121] Several studies focus on environmental exposures to dietary antigens and infectious agents, but evidence is limited.[122,123]

Epigenetic studies on MZ twins concordant for T1D demonstrated significant differences in the epigenome, particularly for DNA methylation content and histone modifications through a trend coined 'epigenetic drift'.[8] The presence of a primitive pancreatic damage can initiate the autoimmune attack and lead to the activation of repair mechanisms like cellular proliferation[124,125] and ultimately influence the integrity of the epigenome. Another mechanism by which epigenetics may play an important role in T1D is by modulating lymphocyte maturation and cytokine gene expression.[126] An example is the differentiation of subtype T helper cells, one of the most complex immune process, which is ruled by epigenetic controls.[127–129] The epigenetic modifications are also important in pancreatic islet cells through the influence of repair mechanisms. Interestingly, glucose and insulin levels are major determinants on the methylation processes that take place in the cell *via* elevated homocysteine and homocysteine remethylation with a concurrent reduced capacity to eliminate homocysteine by transsulfuration. Homocysteine can be thus remethylated to form methionine and then converted to S-adenosylmethionine, the major methyl group donor in cellular methylation reactions.[130]

The establishment and maintenance of methylation patterns of CpG dinucleotides in DNA and histones depend on cellular methyl group metabolism, which is dependent on various nutrients, as in the case of folate.[130] These relations between food and epigenetic mechanisms acquire importance during embryogenesis, intrauterine and perinatal life as demonstrated in animal models[131] and human studies.[132] These changes may well affect the offspring pancreas[133] as in the case of a low-protein diet decreasing islet mass and vascularity.[134,135]

Multiple sclerosis (MS)

MS is an inflammatory chronic disease characterized by myelin destruction followed by a progressive grade of neurodegeneration in multifocal loci called plaque.[136,137] The etiopathogenesis of MS remains largely unknown but the current hypothesis encompasses an immune-mediated damage determined by the activation of immune cells types against self white matter epitopes to develop diffuse plaques in the central nervous system with resulting inflammation. Genetic linkage studies and genome-wide profiling arrays have enabled the identification of several genes significantly associated with MS susceptibility,[138–141] as in the case of MHC.[142] However, the 20–30% concordance rate for MS among MZ twins[143,144] emphasizes the importance of environmental factors in MS pathogenesis possibly *via* epigenetic mechanisms.[142] Tissue damage implies the activation of developmental pathways,[145,146] but in patients with MS these appear to be unregulated in the presence of repair events.[147,148]

There is limited data on epigenetics of MS, but a 30% reduction was reported in the methylation rate of cytosines in CpG islands was found in the white matter of affected central nervous tissue compared to controls.[149] Further evidence on the role of hypomethylation was found at the promoter region of peptidyl arginine deiminase, type II, which is overexpressed in MS and is involved in the citrullination process of myelin basic protein (MBP).[150,151] These data are in agreement with the observation of an increased demethylase enzyme activity in MS.[152] The citrullination of MBP by peptidyl arginine deiminase determines important biologic effects such as to promote protein autocleavage[153] and a resulting increased probability to create new epitopes.[154] Several studies support the importance of citrullination of MBP in modulating the immune response in MS[155] *via* two mechanisms. In fact, citrullination increases the production of immunodominant peptides, due to increased autocleavage of the protein.[153] This process leads to irreversible changes in the biological properties of MBP which becomes more prone to proteolytic digestion and causes myelin instability.[154] These MBP alterations during the early stages of MS may contribute to the sensitization of T cells also by enhancing the autoimmune response[153] leading through a chronic inflammatory response. Most recently, the comparison of multiple epigenetic readouts in CD4$^+$ T cells from MZ twins discordant for MS failed to identify consistent associations,[156] thus possibly

suggesting that broad approaches may not constitute the ideal approach to complex conditions. These negative findings also applied to HLA haplotypes, confirmed MS susceptibility polymorphisms, copy number variations, mRNA and genomic single nucleotide polymorphism and insertion/deletion genotypes, or the expression of approximately 19 000 genes.

The importance of determining the epigenetic bases of MS, similar to other complex conditions, is of particular importance in the search for therapeutic implications, as well represented by the beneficial effects of trichostatin A, a HDAC inhibitor, in MS murine models.[157]

Primary biliary cirrhosis (PBC)

PBC is a chronic immune-mediated cholestatic liver disease characterized by the destruction of the small interlobular bile ducts, leading to portal inflammation, fibrosis and/or cirrhosis.[158]

Similar to most autoimmune diseases, PBC affects primarily women (female/male ratio estimated as 10 : 1) with a peak of incidence in the fifth decade of life. Enhanced awareness of the condition and increased availability of diagnostic tools, in particular serological testing, have led to a more frequent and earlier diagnosis of PBC,[159,160] more commonly at asymptomatic stages.[161]

PBC etiopathogenesis recognizes an important role for genomics, possibly stronger than in other autoimmune disorders.[162,163] Indeed, PBC concordance rate in MZ twins is 63% the highest among autoimmune diseases with the exception of celiac disease.[164]

New approaches are being sought to identify the presence of epigenetic marks which can participate to PBC susceptibility. In fact not only genetic polymorphisms in the first genome-wide studies but also epigenetic impaired mechanism could be involved in the break of self-tolerance. Mitchell and colleagues most recently described the differential expression of two X-linked genes (PIN4 and CLIC2) that were differentially methylated in discordant MZ twins.[165] This is of particular importance based on our previous report of a possible X-chromosome haploinsufficiency.[166]

WHERE WE ARE AND WHAT IS NEXT FOR THE EPIGENETICS OF AUTOIMMUNITY

Most recently, there have been numerous studies to support the importance of epigenetics in the initiation and perpetuation of autoimmunity in specific conditions. In some cases, findings were recapitulated in different conditions, thus supporting the theory of a common theme for the autoimmunologist[1,167] and providing fascinating bases for the geographical pattern of autoimmunity epidemiology (i.e. geoepidemiology).[89,168–170] Over the past years, there has been an enormous development of genome-wide mapping for DNA methylation[171] and histone modifications[26] with novel issues arising[172,173] particularly in terms of environmental epigenetics.[174] Every cell process is permeated by epigenetic regulation, from cancer[175] to autoimmune diseases.[176] The understanding of these mechanisms and the identification of target molecules are expected to lead to new classes of therapeutical molecules, coined 'epigenetic therapies'.[177,178] We foresee that only a common effort between researchers involved in human and experimental autoimmunity and the use of powerful tools such as MZ twins will soon provide fascinating developments in the relatively young field of epigenomics. As an example, epidemiology and basic epigenetics should work together to provide solid associations between environmental factors and DNA methylation or histone changes in patients with autoimmune diseases.

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