

The role of epigenetic mechanisms and processes in autoimmune disorders

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Abstract: The lack of complete concordance of autoimmune disease in identical twins suggests that nongenetic factors play a major role in determining disease susceptibility. In this review, we consider how epigenetic mechanisms could affect the immune system and effector mechanisms in autoimmunity and/or the target organ of autoimmunity and thus affect the development of autoimmune diseases. We also consider the types of stimuli that lead to epigenetic modifications and how these relate to the epidemiology of autoimmune diseases and the biological pathways operative in different autoimmune diseases. Increasing our knowledge of these epigenetic mechanisms and processes will increase the prospects for controlling or preventing autoimmune diseases in the future through the use of drugs that target the epigenetic pathways.

Keywords: twins, concordance, autoimmune disease, nongenetic factors, immune system, epigenetic modifications

Epigenetics and epigenetic mechanisms What do we mean by "epigenetics"?

"Epigenetics" can be broadly defined as events or processes that affect the inheritance of gene activities but do not depend on any changes in DNA base sequences. The activity of genes is dependent largely on whether they are accessible to transcription factors; this is highly regulated by the dynamics of chromatin restructuring.¹ The basic repeating unit of chromatin is the nucleosome, in which ~147 base pairs of negatively charged DNA wrap 1.65 times around a highly positively charged histone protein octamer, consisting of the H2A, H2B, H3, and H4 histone subunits (Figure 1A). The nucleosomes compact further to form the 30 nm fiber, with the H1 histone subunit playing an important role in stabilizing this structure. However, this tightly compacted chromatin structure poses barriers for processes such as transcription and replication, which require that the two strands of DNA come apart temporarily. Epigenetic "marks" on the chromatin play a central role in regulating the structure of chromatin (Figure 1B) and thus the accessibility of DNA for transcription.²

The actual term "epigenetics" was coined by CH Waddington in 1942 to describe the (at that stage, undefined) causal mechanisms by which the genes of a genotype could bring about phenotypic effects;³ for example, the process by which differentiated cells maintain their specialized phenotypes through repeated cell division, with some genes remaining permanently switched on and others permanently switched off, even though all cells of an organism carry the same complement of DNA, was labeled an "epigenetic" control system.⁴ It was not until the 1980s, however, that

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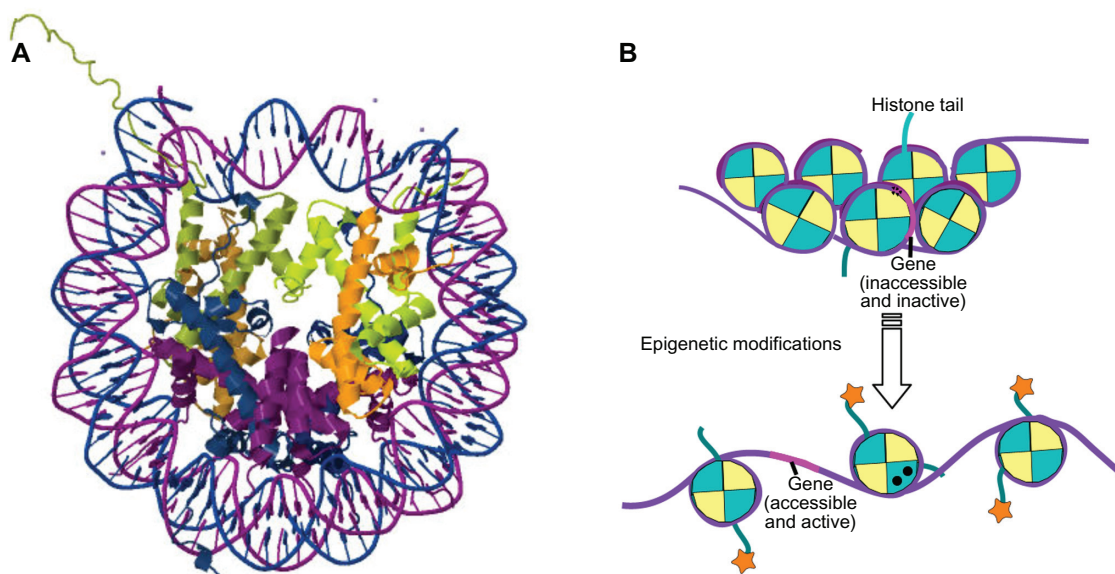


Figure 1 (A) Cartoon derived from the crystal structure (Protein Data Bank ID: 1aoi²⁹⁶) of the histone octamer (H2A, blue; H2B, purple; H3, green; H4, orange) surrounded by 1.65 turns of DNA (~147 base pair fragment). (B) In compacted chromatin, genes (represented in pink) are inaccessible and inactive, with hypermethylation of their promoter regions (▲), and there are few posttranslational modifications to the histones. Epigenetic modifications in the form of demethylation of gene promoter region, posttranslational modification of the histones (★), or swapping of histone subunits (↔), lead to opening up of the chromatin. The gene is then accessible to transcription factors and RNA polymerase.

the term "epigenetics" was applied to a specific chemical modification, namely DNA methylation, that could affect the inheritance of gene activities without any changes in DNA base sequences.⁵ Subsequently, the epigenetic label has also been applied to many histone modifications and other chromatin modifications that regulate transcription and/or replication,^{6,7} including gene silencing and X-inactivation, and to the regulatory actions of noncoding RNAs.⁸ Some investigators have argued that histone modifications per se are not epigenetic,⁹ as they have not been conclusively demonstrated to be self-propagating (heritable); however, as specific histone modifications can induce DNA methylation events,^{10,11} and DNA methylation events affect histone acetylation and histone methylation, there is an indirect heritability to these events.¹² More recently, several authors have attempted to clarify the definition of epigenetics to encompass all that it has come to mean;^{13,14} in this paper we will take a fairly broad definition of "epigenetics" as modifications that do not involve DNA base changes, that play a central role in controlling tissue and signal-specific gene expression, and that are responsible for the determination of gene expression profiles of tissues and cellular subsets.

Epigenetic modifications

Epigenetic modifications act by changing the way that DNA and histones interact in the nucleus, thereby allowing or preventing access by transcription factors and RNA

polymerases, and regulating gene expression. Understanding the processes by which the epigenetic modifications result in a specific outcome is a very topical area of research, but there are still some large gaps in the specifics of how some of these modifications exert their effects.

DNA methylation

DNA methylation is an important epigenetic modification and is of great interest to autoimmunity, as treatment with the DNA methylation inhibitor 5-azacytidine is sufficient to induce autoimmune disease in experimental animals.^{15,16} In mammals, DNA methylation typically involves the attachment of a methyl group to cytosine moieties in CpG dinucleotides (Figure 2) and occurs at ~70%–80% of CpG sites throughout the genome.¹⁷ Methylated cytosine can be deaminated to thymine, either spontaneously or via enzymatic

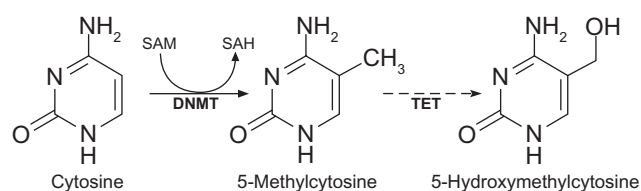


Figure 2 Methylation of cytosines in DNA.

Notes: In the presence of DNA methyl-transferases (DNMTs), methyl groups donated from S-adenosylmethionine (SAM) convert cytosine to 5-methylcytosine. 5-hydroxymethylcytosine can be generated by oxidation of 5-methylcytosine, via the action of the ten-eleven translocation (TET) family of enzymes.

Abbreviation: SAH, S-adenosylhomocysteine.

processes, leading to an underrepresentation (less than a quarter of what would be expected) of CpG sequences in the genome. However, dense clusters of short (~500 bp) CpG-rich regions, known as "CpG islands,"^{18,19} also occur, particularly within the promoter region of genes, upstream from the transcriptional start sites (TSS), as many transcription factors require CpG-rich sites to bind to DNA.²⁰ The majority of CpG islands near TSS are hypomethylated; methylation of promoter CpG islands is usually linked to gene repression,¹² although it is still not clear if methylation precedes compaction of the nucleosomes, or whether it might act to "lock" the compacted nucleosomes.²¹

Intragenic and intergenic CpG islands also occur and appear to be more susceptible to methylation than those in the promoter region.²² It is thought that intragenic CpG islands may in some instances represent alternative TSS that could, for example, be utilized in a highly tissue-restricted fashion.^{21,23} Furthermore, methylation in the gene body can silence repetitive DNA elements, such as retroviral elements and, in contrast to methylation in promoter regions, also appears to actively stimulate transcription elongation.^{19,21} The functional significance of intergenic CpG islands is as yet unclear, although studies have localized at least some of these to promoters of noncoding RNAs,^{24,25} suggesting they may help regulate expression of these molecules.

DNA methylation also appears to play regulatory roles outside of CpG islands. For example, recent studies on colon cancer have described DNA hypo- or hypermethylation of oncogenes or tumor repressors, respectively, at "CpG island shores," which lie up to 2 kb from CpG islands.²⁶ Recently, there have been several reports of regions of the genome that have only 10%–50% of the typical amount of CpG methylation and that appear to be involved in binding of transcription factors or nuclear receptors (such as the glucocorticoid receptor) in a cell-type specific manner.^{27,28} The formation of these "low-methylated regions" (LMRs) has been found to significantly correlate with increased expression of the nearest gene, whereas remethylation of LMRs coincides with reduced expression; this suggests that these LMRs represent distal regulatory regions.²⁷ Interestingly, 5-hydroxymethylcytosine (Figure 2), which is produced by oxidation of 5-methylcytosine,²⁹ and TET1, which belongs to the family of ten-eleven translocation (TET) enzymes that catalyze this oxidative event,³⁰ appear to both be enriched in LMRs;²⁷ as 5-hydroxymethylcytosine has been reported to be present at enhancer regions,^{31,32} its enrichment in the LMRs provides support for the LMRs having a regulatory role.

Because DNA methylation plays such a critical role in regulating gene expression, the mechanisms for establishing, maintaining, and removing methyl groups have also been the subject of intense scrutiny, although there are still some major unanswered questions. DNA methylation of cytosines in mammalian cells occurs through the action of 5-methylcytosine DNA methyltransferases (DNMTs), which transfer a methyl group from a methyl group donor, S-adenosylmethionine, to the fifth carbon of the cytosine residues, converting them to 5-methylcytosines (Figure 2). Five DNMTs have been identified in mammalian cells. It was thought for some time that DNMT1 could, by itself, maintain patterns of DNA methylation during cell replication; however, it is now recognized that ongoing participation from DNMT3a and DNMT3b is also required for methylation maintenance.³³ The latter are also critical for *de novo* methylation.³⁴

It has been reported that DNA methylation can be very dynamic, with some genes undergoing cyclical changes within minutes to hours,³⁵ but the mechanisms by which this type of rapid methylation/demethylation can occur are still not completely understood. Passive mechanisms of demethylation, such as occur when cells divide or when mammals erase genomic methylation patterns in primordial germ cells, are unlikely to account for such rapid cyclical changes in methylation patterns. However, the search for active demethylases has thus far not yielded very reproducible results.³⁶ It is thought that the oxidation of 5-methylcytosine to 5-hydroxymethylcytosine via the action of TET family molecules may play a role in the more rapid cycles of methylation/demethylation, particularly in transcriptional regulatory regions such as enhancers.^{21,37}

Initially, it was thought that methylation of CpG sites caused transcriptional repression by directly interfering with the binding of transcription factors to DNA;³⁸ however, it now appears that this is not the main mechanism by which methylated DNA inhibits gene expression. Instead, the methylated DNA attracts proteins that contain a methylated-DNA binding domain (MBD).^{39,40} MBD-containing proteins provide links between methylated DNA and covalent and non-covalent histone modifications that act to modify chromatin and repress gene activity.⁴¹

Histone modifications and chromatin remodeling

Covalent modifications to the histone protein subunits can be made throughout the proteins; however, the N-termini of the histones, which extrude from the tightly wound chromatin strands, are particularly susceptible

to posttranslational modifications. Such modifications can include acetylation, methylation, ubiquitylation, phosphorylation, and SUMOylation.⁴² Acetylation/deacetylation and methylation/demethylation appear to be of most importance in regulating gene expression. Acetylation (via the action of histone acetyltransferases) of selected lysine residues in the tails of nucleosomal histones removes the positive charge on the lysine amino group that is acetylated, loosening the interaction between DNA and the histone and preventing chromatin from folding into the 30 nm fiber;⁴³ this typically promotes gene expression.^{44,45} In contrast, deacetylation (via histone deacetylases [HDACs]) is generally associated with gene repression. The phosphorylation state of histone H1 also appears to play a role in the folding of chromatin into higher-order structures.⁴⁶ The effects on gene regulation of methylation of lysine or arginine residues in the histone tails depend on both the specific site of the modified residues in the histone tail and the number of methyl groups that are added to these residues.^{47,48} In some cases, the same amino acid of a histone tail can be either acetylated or methylated with varying consequences; for example, lysine at position 9 of histone H3 (H3K9) is acetylated in regions of the genome that are transcriptionally active, but methylated in areas that are transcriptionally silent. Other specific histone modifications with well-defined effects on gene expression include H4 hyperacetylation and trimethylation of H3K4, both of which are present in many active genes.⁴⁹ Common sites of posttranslational histone modifications in H3 and H4 are shown in Table 1, although not all of these are necessarily epigenetic – some will probably be of more relevance to DNA replication and repair.

Acetylation of lysine and methylation of lysine or arginine on histone tails plays a role in orchestrating the recruitment of multiprotein complexes. Some components of these complexes can "read" the histone changes via molecular interactions of the methyl/acetyl chains with structurally conserved protein modular domains that are present in many chromatin regulators and transcription factors and that act to change chromatin structure at target gene loci.⁵⁰ Some of these "readers" are extremely versatile: for example, proteins containing plant homeodomain (PHD) fingers appear to be able to read histone states in a sequence-dependent manner that is modulated positively or negatively by the methylation state of lysine and arginine, as well as by the acetylation state of lysine (reviewed in Sanchez and Zhou⁵¹). Other components of the protein complexes recruited by the histone modifications are involved in chromatin remodeling: these components contain a conserved ATPase domain.⁵²

Remodeling utilizes energy from ATP hydrolysis to mobilize and restructure the nucleosomes and/or replace the histones themselves with histone variants.^{52,53} For example, remodeling complexes of the SWR1 family can replace H2A histones in H2A-H2B dimers with H2A.Z, forming variant nucleosomes with unique histone tails that can potentially bind unique regulatory proteins.⁵⁴ Other remodelers enable movement of nucleosomes along the DNA, thereby exposing new DNA to regulatory factors. Exactly how remodelers mobilize the nucleosome and how different remodeler complexes select which nucleosomes to move and restructure is still imperfectly understood.

Noncoding RNAs

Around 98% of the transcribed human genome does not encode proteins⁵⁵ and recent studies have identified several different types of noncoding RNAs that act posttranscriptionally to regulate many biological processes via their interactions with messenger RNA (mRNA) or DNA.^{16,56,57} Currently, those thought to be of most relevance to autoimmunity are the microRNAs (miRNAs) and long noncoding RNAs (lncRNAs), although it is likely that further investigation will find that at least some of the other noncoding RNA types are also important.

The miRNAs are small molecules of around 22 nucleotides in length, which are believed to regulate the translation of more than 60% of protein-coding genes.^{57,58} In humans, more than 1000 miRNAs have been identified.^{56,59} Translation of mRNA into protein is blocked by human miRNAs primarily by inhibition of translation initiation either via binding of the miRNAs to the 3'UTR⁵⁷ or (less frequently in humans) via induction of mRNA degradation.⁶⁰ Some miRNAs regulate specific individual targets, while others appear to function as master regulators of a process, regulating expression levels of hundreds of genes simultaneously.^{56,57,61} Recent studies suggest that there is strong bidirectional regulation between specific miRNAs and HDACs. For example, downregulation of miR-9 in bone marrow-derived CD19⁺ cells from patients with the B-cell lymphoma Waldenström's macroglobulinemia results in upregulation of HDAC4 and HDAC5 expression levels and aberrant histone acetylation in these cells, and leads to abnormal expression of genes that cause the lymphoma.⁶² Conversely, HDACs that are overexpressed in chronic lymphocytic leukemia block critical apoptosis-related miRNAs in the malignant B cells, resulting in pro-survival signals.⁶³

A fast-expanding area of research involves the role of lncRNAs in epigenetics. The definition of lncRNAs is complicated by the observation that lncRNAs often overlap with, or are

Table I Posttranslational modifications to tails of histones H3 and H4

Position	Amino acid*	Posttranslational modification	Site where active (if known)**	Likely outcome on transcriptional state of chromatin (if known)
Histone H3				
2	R	Methylation	GB	
3	T	Phosphorylation		
4	K	Mono-methylation	E, P	Activation
		Di-methylation	E, P	Activation
		Tri-methylation	P	Activation > repression
		Acetylation	P	Activation
8	R	Methylation		
9	K	Mono-methylation	P	Activation
		Di-methylation	E, GB	Repression
		Tri-methylation	E, GB, P	Repression
		Acetylation	P	Activation
10	S	Phosphorylation		
14	K	Acetylation		Activation
17	R	Methylation		? Activation
18	K	Acetylation	P	Activation
23	K	Acetylation	GB, P	Activation
26	R	Methylation		
27	K	Mono-methylation	GB	Activation
		Di-methylation	GB	Repression
		Tri-methylation	P	Repression > activation
		Acetylation	E, LMR, P	Activation
28	S	Phosphorylation		
36	K	Tri-methylation	GB	Activation
		Acetylation	P	Activation
56	K	Acetylation		Activation
79	K	Mono-methylation	GB	Activation
		Di-methylation	GB	Activation
		Tri-methylation	GB	Activation or repression
Histone H4				
1	S	Phosphorylation		
3	R	Methylation	GB	
5	K	Acetylation	P	Activation
8	K	Acetylation	P	Activation
12	K	Acetylation	P	Activation
16	K	Acetylation	P	Activation
20	K	Mono-methylation	P	Activation
		Tri-methylation	GB	Repression

Notes: *Single-letter amino acid code: K, lysine; R, arginine; S, serine; T, threonine. **Information in this table has been assembled from references 23, 48, 50, and 228–234. ? indicates that this modification probably leads to activation, but it is not yet proven.

Abbreviations: E, enhancer; GB, gene body; LMR, low-methylated region; P, promoter.

interspersed between, multiple coding and noncoding transcripts; however, typically, they are more than 200 nucleotides in length and probably make up the largest portion of the mammalian noncoding transcriptome.⁶⁴ The lncRNAs appear to be able to recruit chromatin remodeling complexes to specific loci⁶⁵ and to regulate gene expression at the level of both transcription^{66,67} and posttranscriptional processing.^{64,68}

Intersection of epigenetic pathways: X marks the spot

One of the best known processes where the various epigenetic pathways and mechanisms intersect is in the regulation of the

X chromosome: normal females possess two X chromosomes and, in any given cell, one chromosome will be active (X_a) and one will be inactive (X_i),⁶⁹ whereas, in normal males, the X chromosome will remain active. However, during reproduction in mammals, the X chromosome undergoes its own cycle of inactivation and reactivation. The paternally derived X chromosome (X^p) is imprinted in pre-implantation female embryos, leading to X^p inactivation.⁷⁰ At implantation, the X^p is reactivated and then, in the embryo proper (when there are ~80–120 cells), X chromosome inactivation occurs randomly in each cell of the embryo,⁷¹ so that all cells derived from each of these initial cells will maintain the same X-inactivation

pattern. Thus, females are mosaics of two cell populations with respect to X-linked gene expression, one expressing the paternally derived genes and one expressing the maternally derived genes.

The X-inactivation center on each X chromosome contains the 17 kb lncRNA, X-inactivation specific transcript (*Xist*), which is necessary and sufficient for inactivation of the chromosome.^{72,73} *Xist* is involved in the transient imprinting of the X^P during embryogenesis.^{74,75} In addition, during the inactivation process, the future Xi dramatically increases *Xist* RNA production and the *Xist* RNA spreads out from the X-inactivation center to progressively coat the chromosome;⁷³ in contrast, the future Xa ceases to express *Xist*. The silencing of genes along the Xi occurs soon after coating by *Xist* RNA; *Xist* appears to be crucial for initiating silencing but has a minor role in maintaining the Xi. Silence is maintained by packaging the Xi into densely packed, transcriptionally inactive heterochromatin, and is associated with high levels of DNA methylation, low levels of histone acetylation, low levels of histone H3K4 and H3R17 methylation, and high levels of histone H3K9 methylation.⁷⁶ Additionally, variants of histone H2A are exclusively found on nucleosomes along the Xi.⁷⁶ Thus, multiple layers of epigenetic modifications are involved in the process of X chromosome inactivation and silencing and they operate cooperatively to regulate gene expression. Given the predominance of autoimmunity in females,^{77–79} it is pertinent to question whether some of these mechanisms may fail in autoimmunity. This will be discussed in subsequent sections.

Studies of monozygotic (MZ) twins support a role for epigenetics in the development of autoimmunity

The etiology of the majority of autoimmune diseases remains obscure; however, for most, it appears that disease arises

because of immune-, gene-, and environment-related effects. Evidence from studies of MZ twins shows that the penetrance of autoimmunity is typically in the order of only 20%–30% (Table 2), suggesting that a particular DNA nucleotide sequence alone is not sufficient for development of disease. For some of these diseases, widely varying concordance rates have been reported; it appears that factors such as the latitude of the country where the study participants lived^{80,81} and the age of disease onset in the proband⁸⁰ can influence the concordance rate, suggesting that environment and aging are major modifiers of the purely genetic effects. In addition, the number of years after diagnosis (in the proband) that the study was done appears also to be a determining factor in the concordance rate. For example, when MZ twin pairs initially discordant for type 1 diabetes (T1D) were followed over many years, the percentage of the initially unaffected twins who subsequently developed T1D increased at a slow rate until the age of 40, but then jumped from 25% at 40 years of age to over 60% at 60 years of age,⁸² suggesting that, during the fifth or sixth decades of life, external and/or internal changes negate the protective effect that has prevented the initially healthy twin from developing disease up until that point. This would be most consistent with a role for epigenetic effects as modifiers of autoimmune disease penetrance, although clonal mosaicism that increases with age may also play a role.^{83,84}

To investigate the role of epigenetics, several studies have looked at whether there are differences in DNA methylation in MZ twins discordant for autoimmune disease. The evidence is variable. Javierre et al⁸⁵ found DNA methylation changes in MZ twins discordant for systemic lupus erythematosus (SLE), but not for rheumatoid arthritis (RA) or dermatomyositis. In studies of female twin pairs discordant for multiple sclerosis (MS), Baranzini et al⁸⁶ found few differences between female MZ twin pairs with respect to either DNA sequences, DNA methylation, or RNA sequences;

Table 2 Autoimmune diseases, showing female:male ratios of patients and concordance rates in monozygotic (MZ) twins

Disease	Target	Female:male ratio	Concordance in MZ twins
Multiple sclerosis	Central nervous system myelin	1.9–4.3:1 ^{140,235,236}	14%–33% ^{81,237–240}
Type 1 diabetes mellitus	Beta-islet cells of the pancreas	0.5–0.8:1 ^{241,242}	13%–60% ^{82,243–245} affected by insulin genotype; ²⁴⁴ latitude ²⁴⁵
Systemic lupus erythematosus	Cell nucleus	8.7–13.1:1 ²⁴⁶	11%–33% ^{85,247–249}
Rheumatoid arthritis	Joints	2.7:1 ²⁵⁰	12%–15% ^{251–253}
Graves' disease	Thyroid	3.5:1 ²⁵⁴	17%–35% ^{255,256}
Primary biliary cirrhosis	Liver	9:1 ²⁵⁷	63% ²⁵⁸
Psoriasis	Skin	0.8–1.1:1 ^{242,259}	35%–70% ^{260,261}
Myasthenia gravis	Acetylcholine receptors	2:1 ²⁶²	35% ²⁶³
Ankylosing spondylitis	Joints	1:3 ²⁶⁴	40%–80% ²⁶⁵

however, because only extreme methylation differences (a change of at least 60%) were investigated in this study, and only far smaller differences have been identified in some other studies using nonmalignant tissues,^{87–89} potential variation could have been missed. In MZ twin pairs discordant for psoriasis, differences in DNA methylation between unaffected and affected twins were correlated with differences in gene expression, particularly in CD4⁺ T cells.⁹⁰ A recent study looked for methylation differences in CD14⁺ monocytes from MZ twin pairs discordant for T1D and identified 132 different CpG sites at which the direction of the intra-MZ pair DNA methylation difference significantly correlated with the diabetic state.⁹¹ Of course, DNA methylation is not the only epigenetic change that might explain the lack of concordance in MZ twin pairs, but as yet there are few studies investigating other potential mechanisms. Furthermore, most of these studies only investigated small numbers of twin pairs and most used peripheral blood containing multiple cell types (and therefore multiple epigenomes) as the source of DNA, which could skew methylation differences,⁹² making it difficult to draw global conclusions from the results.

Evidence for epigenetic modifications in autoimmune diseases

Each autoimmune disease involves at least two major players, the immune system, which is the effector of the autoimmune damage, and the target organ, which can have variable degrees of resistance to autoimmune damage. Epigenetic modifications to either the immune system or the target organ could play a role in disease development. In the following section, we will review some of the modifications that have been found thus far, but major questions remain unanswered, such as: Are the same epigenetic modifications seen in all patients with the same clinical phenotype? How do the epigenetic effects interact with polymorphisms in autoimmune susceptibility genes? Is there a threshold in the number/type of epigenetic modifications that need to occur for disease to become manifest?

Epigenetic modifications in the immune system

Numerous recent studies clearly indicate that there is epigenetic control of major immune cell functions, such as hematopoietic lineage choice, antigen-receptor rearrangement and allelic exclusion, and immune responses to pathogens.^{93–105} For example: epigenetic processes govern the differentiation of T helper cells and their lineage stability,^{93,105} exposure of dendritic cells to oxidized phospholipids

can change the phenotype of the cells through epigenetic mechanisms,¹⁰⁶ and epigenetic processes control the production of antibodies by B cells.¹⁰⁷ These immunological developmental events that are regulated by epigenetic mechanisms could possibly be altered to promote autoimmunity. In this section, we will focus on some of the epigenetic modifications that could specifically affect susceptibility to, or development of, autoimmune diseases.

Epigenetic control of immune tolerance

One of the major mechanisms in the control of development of autoreactive immune cells is induction of T cell central tolerance in the thymus. Central tolerance against many autoantigens is regulated by the autoimmune regulator (AIRE) protein, which promotes expression of tissue-specific antigens in thymic medullary epithelial cells. AIRE contains PHD fingers, which bind to methylated histone H3 and provide a link between the status of histone modifications and the regulation of tissue-specific antigen expression in the thymus. In an animal model with a targeted mutation within one of these PHD fingers, there was decreased binding of AIRE to methylated histone H3, a generalized dampening of AIRE's transcriptional impact, and development of autoimmunity.¹⁰⁸

Another potential mechanism by which epigenetic mechanisms could affect central tolerance is via the action of the lncRNA, growth-arrest-specific 5 (*GAS5*). *GAS5* sensitizes cells to apoptosis by regulating the activity of glucocorticoids, is present in T cells,¹⁰⁹ and has been linked to increased susceptibility to autoimmune disorders, including SLE.¹¹⁰ Positive selection of T cells in the thymus is postulated to be dependent on the counter-interaction between glucocorticoid receptor- and T cell receptor-induced death signals.¹¹¹ Modulation of *GAS5* expression levels could regulate selection of T cells through the glucocorticoid receptor, thereby providing resistance to apoptosis, allowing escape of self-reactive T cells, and potentiating development of autoimmunity.

There is evidence that miRNAs also play a major role in induction of central tolerance. For example, the affinity of maturing T cells for antigen during thymic development plays a large role in determining their fate: there appears to be a strong correlation between the sensitivity of the T cells to antigen and levels of miR-181a, which is dynamically regulated during T cell maturation in the thymus.¹¹² In addition, recent studies have identified a selection of miRNAs that have known functions in thymopoiesis and which are differentially regulated in the thymus following stress; this

dysregulation has the potential to alter T cell repertoire selection and the formation of naive T cells.¹¹³

In the periphery, some of the best-studied mediators of tolerance are the CD4⁺ Foxp3⁺ regulatory T cells (Tregs). Foxp3 is known to form complexes with histone acetyltransferases (Tip60 and p300) or HDACs (HDAC7); these complexes then epigenetically modulate target gene expression via histone or Foxp3 acetylation or deacetylation.^{114,115} Recently, it was shown that mice in which Foxp3 failed to associate with Tip60 and HDAC7 had altered Foxp3-dependent transcription and epigenetic modifications, causing insufficiency in natural Tregs and impaired development of inducible Tregs.¹¹⁶ It has been reported that Foxp3 also represses the activity of SATB1, a genome organizer that regulates chromatin structure and gene expression, leading to loss of suppressive function in the Tregs and establishment of effector functions.¹¹⁷ Foxp3 not only acted directly as a transcriptional repressor of SATB1 but also indirectly through the induction of miRNAs that bind the SATB1 3' untranslated region. Other studies have also highlighted the role that miR-155 plays in Treg development and that miR-146 and the miR-17-92 cluster play in Treg function (as recently reviewed in Zhou et al, 2011).¹¹⁸

Other cells involved in maintenance of peripheral tolerance include natural killer T cells and regulatory subsets of dendritic cells. Several reports of miRNA- or HDAC-mediated regulation of these subsets of cells exist, although most have not specifically addressed the issue of how levels of these molecules change when peripheral tolerance is compromised.^{118,119} Another study has suggested that programmed death-1, a negative regulatory molecule expressed on activated CD4⁺ and CD8⁺ T cells, maintains immune homeostasis and self-tolerance through a miRNA (miR-21) signaling cascade.¹²⁰

The studies looking at epigenetic control of tolerance have probably only touched the tip of the iceberg with respect to the complexity of the levels of control and likely mechanisms; however, they do provide support that this is one way in which epigenetics could affect autoimmunity.

Epigenetics and human leukocyte antigen (HLA)-disease associations

A large number of autoimmune diseases are strongly linked to carriage of specific HLA (particularly HLA class II) molecules; interestingly, however, HLA linkage for some autoimmune diseases varies around the globe. In MS, for example, linkage is with DRB1*1501 in Caucasian populations and DRB1*0301, DRB1*0405, and DRB1*1303 in Sardinia, but DRB1*07 in continental Italy and DRB1*13 in

Israel. It has been shown that coordinated changes of histone modifications and HDAC mobilization regulate the induction of HLA class II genes¹²¹ and proposed that the different HLA associations observed in MS patients across the globe are a reflection of specific environmental factors influencing epigenetic marks on liable haplotypes, which affect the expression or function of class II genes and permit the MS pathogenic cascade.¹²² In MS, there is also some evidence of epigenetic modification of HLA molecules contributing to the inheritance of disease susceptibility, particularly transmission from mothers to offspring.^{123,124}

Other diseases have been less well studied, but in autoimmune diseases where HLA linkage is important, epigenetic modification of the HLA molecules could possibly modify the HLA linkage. In diseases such as ankylosing spondylitis, where there is strong HLA linkage and good concordance between MZ twins, this is less likely to play a role.

Abnormal epigenetic marks on peripheral blood mononuclear cell (PBMC) subsets

There have been multiple reports of altered levels of epigenetic marks in PBMCs and subsets of these, particularly B and T cells, from patients with autoimmune disease compared with healthy controls: these are summarized in Table 3. Some alterations appear to be relatively disease specific, whereas others may relate more to the autoimmune phenotype. PBMCs from patients with SLE have been studied extensively and recently reviewed:^{125,126} in brief, there is global hypomethylation of B and T cells, alterations in DNMT expression and in histone acetylation, particularly affecting genes related to apoptosis, and changes to levels of specific miRNAs. The mechanisms by which these changes in epigenetic marks modulate specific cellular processes are currently the subject of intense research interest.

Epigenetic modifications in the target organ

As noted, epigenetic changes in some autoimmune diseases are particularly notable in cells of the immune system; however, changes in the target organ could also render the organ more susceptible to autoimmune attack. Such failure of target-organ resistance could play a role in the development of autoimmune disease.^{127,128} What is still unknown is whether epigenetic changes in both the immune system and the target organ are required for development of autoimmune disease, whether changes in one are more likely to occur than changes in the other (eg, whether organs/cell systems that turn over more rapidly are more likely to be affected), or whether the

Table 3 Abnormal epigenetic marks on peripheral blood leukocytes in autoimmune diseases

Cell type	Disease	Epigenetic modification	
B cells	SLE ^{190,266–269}	Global DNA hypomethylation ↓ DNMT1 ↓ HDAC1 CD5 (E1B isoform) demethylation Demethylation of HERV LINE-1	
CD4 ⁺ T cells	RA ²⁷⁰	↑ miR-146	
	SLE ^{271–275}	Global DNA hypomethylation ↓ DNMT1 ↓ DNMT3a ↑ MBD1 ↑ MBD3 ↑ MBD4 ↓ HDAC2 and HDAC7 Global H3 and H4 hypoacetylation and hypermethylation, ↑ miR-126 Demethylation of <i>CD11A</i> , <i>CD70</i> and <i>CD154</i> (in women) Demethylation of <i>CD70</i> promoter regulatory elements ²⁷⁶	
	Sjögren's ²⁷⁶	Global DNA hypomethylation <i>CD70</i> promoter demethylation ↓ DNMT1, ↓ MBD3, ↓ MBD4	
	Scleroderma ^{273,277}	↑ MBD2 ↑ MeCP2	
	Dermatomyositis ²⁷³	Global DNA hypomethylation ↑ miR-223 Demethylation of <i>IFNG</i> and <i>FOXP3</i> in CD4 ⁺ T cells infiltrating the joint ²⁸⁰	
	RA ^{49,278,279}	↑ miR-17-5p and miR-193a ↑ miR-326 (in Th17 cells) ↓ miR-497, miR-1 and miR-126 Demethylation of <i>FOXP3</i>	
	MS (relapsing-remitting) ^{280,281}	Demethylation of <i>CD40L</i> promoter	
	Primary biliary cirrhosis ²⁸²	Global DNA hypomethylation Perforin promoter demethylation	
	CD8 ⁺ T cells	SLE ^{15,283}	Differential methylation of 132 different CpG sites
	Monocytes	T1D ⁹¹	↓ miR-146, miR-17-5p, miR-112, miR-141, miR-184, miR-196a miR-383, miR-409-3p ↑ miR-21, miR-61, miR-78, miR-142-3p, miR-189, miR-198, miR-298, miR-299pp, miR-342
PBMCs	SLE ²⁸⁴	↑ miR-146, miR-155, miR-132, miR-16	
	RA ⁴⁹	Global histone hyperacetylation Demethylation of <i>CD21</i>	
	T1D ²⁸⁵	Altered histone methylation	

Abbreviations: DNMT, DNA methyl-transferase; HDAC, histone deacetylase; HERV LINE-1, human endogenous retroviral element long interspersed repetitive element 1; MBD, methylated-DNA binding domain; MeCP2, methyl CpG binding protein 2; MS, multiple sclerosis; PBMCs, peripheral blood mononuclear cells; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; T1D, type 1 diabetes mellitus.

type of autoimmune disease (ie, organ specific vs systemic) correlates more with epigenetic changes in the target organ than in the immune system.

During the last 2–3 years, a substantial body of evidence has accumulated to suggest that epigenetic changes in the target organ are important in some autoimmune diseases. For example, in synovial tissues from patients with RA, there is hypomethylation of DNA,¹²⁹ decreases in levels of HDAC1 and HDAC2 and hyperacetylation of histones H3 and H4,¹³⁰ and hypomethylation of histone H3 at lysine 9. Similarly,

in MS patients, compared with healthy individuals, there is hypomethylation of DNA from normal-appearing central nervous system white matter, but not from tissue in the thymus, demonstrating that the target organ alone can show epigenetic changes.¹³¹ In anti-neutrophil cytoplasmic antibody vasculitis, there is upregulation of the target antigen due to perturbation of epigenetic gene-silencing mechanisms.¹³² SLE is a special case when considering the target organ, as the target organ of SLE, the cell nucleus, is also the site of the epigenetic effects. Multiple studies over a number of years

have shown that modifications to both DNA and histones can affect their antigenicity. Apoptotic hypomethylated DNA and posttranslationally modified histones are known to be major targets of autoantibodies in SLE.^{133–136} Table 4 lists changes in epigenetic marks that are seen in the target tissue of some autoimmune diseases. In some cases, the interpretation of the target-tissue data might be made more difficult by the presence of immune cells or other inflammatory mediators within the target organ.

What stimuli might induce epigenetic changes that lead to the development of autoimmunity?

In most cases, the nature of the specific stimuli that lead to the epigenetic changes seen in patients with autoimmune disease remain undefined, but could include many diverse stimuli, both external (eg, diet, exposure to sunlight, environmental chemicals, toxins, and drugs/pharmaceuticals) and internal (eg, aging, stress, sex hormones). A complicating factor in studies of stimuli of epigenetic changes is that, while some epigenetic effects are manifested in the generation of patients that are exposed to the modifying agent, in other cases, it appears that disease occurs predominantly one or two generations after the exposure.¹³⁷

It is also of interest to consider how different stimuli might differentially affect males and females, given the higher incidence of autoimmunity in females (Table 2).^{77–79} For epigenetic modifications to explain sexual dimorphism in autoimmunity, it is necessary to suppose that epigenetic factors are more commonly encountered in one sex than in

the other, or that members of one sex are more vulnerable than the other: this is speculative. However, in view of the increase in some autoimmune diseases among women, but not men, over the last 100 years,^{138–142} and the major societal changes that have particularly affected women over that time, it is an attractive hypothesis.

Effects of external exposure to agents that induce epigenetic changes

Diet and nutrition

The diet provides the methyl donors (methionine, choline) and cofactors (folic acid, vitamin B12 and pyridoxal phosphate) essential for DNA and histone methylation.¹⁴³ It is now well recognized that susceptibility to adult-onset chronic disease is influenced by persistent adaptations to prenatal and early postnatal nutrition.^{144,145} Furthermore, there are also reports of diet-induced epigenetic changes in the adult state. For example, dietary components such as alcohol, vitamin B6, and vitamin A have been linked to DNA methylation within the gastrointestinal tract and development of colorectal cancer^{146–149} and diets such as the "epigenetic diet" have been popularized as a means of controlling cancer via epigenetic modification.¹⁵⁰

It has been proposed that epigenetic links between nutrition and autoimmunity may well contribute to the epidemiology observed for numerous autoimmune diseases;¹⁵¹ however, while studies in animals using arbitrarily chosen dietary elements tend to support this proposal, human data from real-life clinical settings or randomized clinical trials remains inconclusive at present. There are, however,

Table 4 Changes in epigenetic marks seen in the target tissue of some autoimmune diseases

Disease	Tissue	Epigenetic modification
SLE	Cell nucleus	Tri-acetylated histone H4, ubiquitinated histone H2A, and acetylated histone H2B are targets of autoantibodies from many patients ^{135,136,286}
RA	Synovial tissue	DNA demethylation ¹²⁹ ↑ miR-155, miR-146a, miR-203 ^{287,288} ↑ levels of histone acetyltransferases ¹³⁰ Hypermethylation of death-receptor 3 (DR3) ²⁸⁹ Demethylation of HERV LINE-1 ²⁹⁰
T1D	Pancreas	Insulin promoter regulated by DNA methylation
Primary biliary cirrhosis	Liver	↓ miR-122a and miR-26a and ↑ miR-328 and miR-299-5p ²⁹¹
Sjögren's	Salivary glands	Altered levels of miR-146a and miR-155 ²⁹²
Psoriasis	Skin	Altered levels of miR-31, miR-203 and miR-142-3p ²⁹³ Altered DNA methylation patterns ²⁹⁴ Demethylation of <i>SHP-1</i> promoter ²⁹⁵
MS	CNS white matter	Hypomethylation of <i>PAD2</i> (causes ↑ MBP citrullination) ³¹ Histone modification of <i>PAD4</i> (causes ↑ MBP citrullination) ²⁹⁶
Scleroderma	Fibroblasts	Aberrant methylation of <i>FLII</i> (causes ↑ collagen synthesis) ²⁹⁷

Abbreviations: CNS, central nervous system; HERV LINE-1, human endogenous retroviral element long interspersed repetitive element 1; MBD, methylated-DNA binding domain; MS, multiple sclerosis; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; T1D, type 1 diabetes mellitus.

suggestions that obesity may predispose to autoimmune disease.¹⁵² In MS, for example, either maternal obesity and diabetes¹⁵³ or teenage obesity¹⁵⁴ may predispose to later MS; increased lipid levels are associated with worse outcome;¹⁵⁵ and increased levels of the adipose-derived hormone, leptin, are associated with decreased numbers of Tregs in patients with MS.¹⁵⁶ There is much evidence to support epigenetic regulation of numerous genes, including many immune-related genes, as a consequence of obesity,¹⁵⁷ although such studies are thus far lacking in obese individuals with autoimmune disease.

Environmental exposures

There is considerable evidence that prevalence rates for autoimmune diseases, including MS, T1D, RA, dermatomyositis, and polymyositis,^{158–161} increase as latitude increases; thus, it is thought that increased exposure to sunlight and ultraviolet radiation (UVR) in areas of lower latitude might be protective, possibly via vitamin D,¹⁶² although recent studies suggest that the protective effects of UVR are not necessarily dependent on vitamin D.¹⁶³ UVR is known to induce multiple epigenetic changes, many of which are immunosuppressive, including hypermethylation of numerous gene promoters, phosphorylation of histone H3, and acetylation of histones H3 and H4.^{164,165} A possible explanation for reduced autoimmunity in men compared with women would be that, generally, males have more sun exposure and use less sun protection than females;¹⁶⁶ however, while females may have less UVR exposure, there could also be biological differences between males and females in response to UVR, as there are gender differences in the degree of carcinogenesis and inflammation after ultraviolet exposure in mice.¹⁶⁷ With respect to vitamin D, it is now known that vitamin D controls the transcription of many genes through multiple epigenetic effects via vitamin D response elements.¹⁶⁸ In addition, there appear to be gender differences in the effects of vitamin D on gene expression;¹⁶⁹ it has been shown, for example, that higher levels of vitamin D are associated with a lower incidence of MS only in women.¹⁷⁰

There are many reports of "outbreaks" of autoimmunity, which tend to occur in areas where there is increased exposure to heavy metals.^{171,172} It has been found that nickel, cadmium, lead, aluminum, mercury, and arsenic (all of which are widespread environmental contaminants) can exert immunomodulatory and toxic effects through epigenetic mechanisms such as global changes to DNA methylation, histone modifications, and changes in levels of miRNAs.^{173,174}

Another common environmental exposure that has been linked to increased prevalence of many autoimmune diseases is tobacco smoke.¹⁷⁵ In addition to nicotine, smokers are exposed to over 6000 other chemicals generated by the burning tobacco, many of which are known to be antigenic, cytotoxic, mutagenic, or carcinogenic. A recent study suggests that maternal smoking can deregulate placental methylation in a CpG site-specific manner that correlates with meaningful alterations in gene expression, particularly along oxidative stress pathways.¹⁷⁶

Drugs/pharmaceuticals

Several drugs, most notably procainamide and hydralazine, are known to cause increases in antinuclear antibodies in most people and lupus-like symptoms in some individuals.¹⁷⁷ Both procainamide and hydralazine induce hypomethylation of DNA, but via different pathways: procainamide is a competitive inhibitor of DNMT1¹⁷⁸ whereas hydralazine inhibits DNMT1 upregulation during mitosis by blocking the extracellular signal-regulated kinase (ERK) signaling pathway at PKC δ .¹⁷⁹ Chemicals in cosmetics have also been suggested to act as a trigger for primary biliary cirrhosis,¹⁸⁰ but the exact mechanisms by which these might act have not yet been elucidated, and thus far there are no studies regarding cosmetics and other autoimmune diseases.

Chemicals that mimic the effects of estrogen (eg, phytoestrogens or drugs such as diethylstilbestrol) are known to exert major epigenetic effects during fetal development,^{181–183} with the dose and timing of exposure in relation to the developmental age of the fetus markedly affecting the outcomes. Thus far, evidence linking these to autoimmune disease is equivocal,¹⁸⁴ although studies are limited. Another possibility that has been considered is that exposure to the oral contraceptive pill might have influenced increasing female incidence of autoimmune disease over the last 30–40 years. Recent studies show that females using the oral contraceptive pill have lower global DNA methylation levels than females who do not use oral contraceptives;¹⁸⁵ however, no specific link between the incidence of autoimmune disease and use of oral contraceptives has yet been identified. There have been reports of worsening of autoimmune disease following assisted reproduction treatment (ART),¹⁸⁶ but so far no clear evidence that children conceived through ART have an increased risk of developing autoimmunity (although the oldest of these children are only now in their early thirties). ART generally involves the use of hormone therapy (with gonadotropin-releasing hormone agonists, follicle-stimulating hormone, luteinizing hormone, human chorionic

gonadotropin, or progesterone) to induce ovulation or to assist in implantation. Recent studies suggest that ART is associated with lower mean methylation at CpG sites in the placenta and higher mean methylation at CpG sites in cord blood, on both imprinted and non-imprinted genes;^{187,188} whether or not these observations will translate to increased levels of autoimmunity in children conceived in this manner remains to be seen. No studies have specifically addressed the issue of whether ART might induce detrimental epigenetic changes in mothers who already have an autoimmune disease.

Retroviruses

Human retroviruses have been linked to multiple autoimmune diseases for many years,¹⁸⁹ although absolute proof that they cause disease is still lacking. However, a large part (~8%) of the human genome is comprised of human endogenous retroviral elements (HERVs) that have incorporated into the primate genome over millions of years. Normally, HERVs are repressed by DNA methylation and other mechanisms; however, it has recently been reported that there is hypomethylation of the HERV long interspersed repetitive element 1 in B and T cells of SLE patients.¹⁹⁰ This could lead to expression of retroviral proteins in the lymphocytes, with potential consequences for the integrity, physiology, and immune function of these cells and subsequent development of autoimmunity.

"Internal" factors that could lead to epigenetic changes of relevance to autoimmunity

Aging

Aging is associated with changes in patterns of gene expression; whether these changes are due primarily to epigenetic alterations or genetic alterations (ie, accumulated DNA damage) is not clear, but it is feasible that epigenetics accounts for at least part of the change. Early studies in mammals found age-associated increases in methylation of CpG islands¹⁹¹ and of histone H4K20;¹⁹² both of these epigenetic marks correlate with transcriptional repression. More recent studies of twins have shown that, although MZ twins are epigenetically indistinguishable during the early years of life, older MZ twins exhibit many differences in their overall content and genomic distribution of methylated DNA and histone acetylation and their gene-expression portrait.^{193,194} In a genome-scale study of epigenomic dynamics during normal human aging, Rakyan et al¹⁹⁵ identified aging-associated differentially methylated regions, which became hypermethylated with aging, predominantly at bivalent chromatin domain promoters. Bivalent

chromatin domains, which contain two methylation sites with conflicting output (H3K4 and H3K27 – see Table 1) are characteristic of pluripotent cells, but methylation of one site dominates as the cells differentiate.¹⁹⁶ This is of interest, as Polycomb-group proteins catalyze the methylation of H3K27, and it has also been reported that Polycomb-group protein target genes are far more likely to become methylated with age than nontargets.¹⁹⁷ Since most autoimmune diseases do not develop until the third or fourth decade of life (with notable exceptions such as T1D), accumulation of epigenetic changes with aging may provide the impetus for the onset of disease in susceptible individuals.

Stress

Physical and psychological stresses have been suggested as being potential modulators in the development of autoimmune disease.¹⁹⁸ Hypomethylation of promoter regions of genes encoding glucocorticoid receptors and brain-derived neurotrophic factor have been reported fairly frequently in offspring whose mothers experienced stressful events during pregnancy. Adolescents whose parents experienced adversity during the adolescent's infancy or preschool years also showed differences in the numbers of methylated CpG sites compared with controls, although these differences were not found to be associated with specific genes.¹⁹⁹ Interestingly, maternal stressors in infancy, but paternal stressors in the preschool years, are most strongly predictive of differential methylation and the patterning of such epigenetic marks varies by the child's gender, with girls showing higher levels of differentially methylated CpG sites than boys.¹⁹⁹

Quality of early maternal care has also long been acknowledged to have long-term repercussions during the lifetime of an individual. Studies in rats have shown that, in the absence of appropriate maternal nurturing, there is less methylation of the gene encoding the glucocorticoid receptor in the hippocampus, resulting in overexpression of the receptor in later life.²⁰⁰ The implication is that the glucocorticoid-mediated stress-response pathway is epigenetically fixed at the level of gene transcription.

Effects of exposure to estrogen

As noted earlier, chemicals that can mimic the effects of estrogen can exert major epigenetic effects during fetal development; similarly, levels of naturally occurring estrogens in the mother during pregnancy can also epigenetically influence fetal development, particularly in the developing brain.²⁰¹ Almost all of the papers looking at the epigenetic modifying effects of estrogens consider them in terms of effects on the

fetus; surprisingly little work has been reported regarding the epigenetic effects on the mother of the greatly elevated levels of estrogens during pregnancy.²⁰² However, the state of being pregnant has short- and long-term beneficial effects on some females with autoimmune disease. In MS, for example, there are reports of favorable long-term effects of childbirth on the course of MS, particularly if the child was born after the onset of MS,^{203–205} and women who have never had children are reported to take a shorter time to reach a higher level of disability than are women who have had children at any time.²⁰³ While these studies could simply indicate that women with more aggressive disease are just less inclined to have children, another possibility is that pregnancy induces long-term protective epigenetic effects in the mother. To understand how/if epigenetic effects of estrogen or other pregnancy-related hormones might help to improve autoimmunity, it will first be necessary to determine the epigenetic marks associated with these hormones in normal healthy cells and organs and compare these with the same patterns in diseased or dysfunctional ones. This information is still lacking.

Exercise

Exercise has numerous health benefits and has effects on the immune system, where it has been shown that exercise in low levels is anti-inflammatory.²⁰⁶ Anecdotal evidence suggests a beneficial role of exercise in autoimmunity, although data from controlled trials is scarce.²⁰⁷ It has been reported that exercise can alter gene expression in skeletal muscle through epigenetic mechanisms, including gene-specific DNA hypomethylation,²⁰⁸ and increased acetylation of H3K36, a site associated with transcriptional elongation, combined with export from the nucleus of HDAC4 and HDAC5 (thereby removing their transcriptional repressive function).²⁰⁹ Although these observations are from small numbers of individuals, and some of the effects reported are relatively small, they suggest that at least some of the effects of exercise may come about via epigenetic modifications. If this were confirmed, it would be plausible that many of the effects of exercise in other tissues, including the immune system, also occur through epigenetic modifications.

Epigenetic modification to the X chromosome

X-linked genes are typically unmethylated (active) in men, while women have one methylated (Xi) and one unmethylated (Xa) gene. It has been found that some genes on Xi are demethylated in females with autoimmune disease; this has been studied most thoroughly in SLE.²¹⁰ The consequence of this appears to be

that females can have elevated levels of expression of molecules encoded on the X chromosome, due to gene dosage irregularities. In support of this, it has been shown that treatment of CD4⁺ T cells with the DNMT inhibitor 5-azacytidine leads to elevated levels of CD40 ligand (CD154 – which is encoded on the X chromosome) on T cells from females but not males.²¹¹ There are observations of elevated levels of CD154 in a multitude of autoimmune disorders, including SLE, autoimmune thyroid disease, T1D, inflammatory bowel disease, psoriasis, MS, and RA. Although the gender of patients has not always been specified in these studies, the results suggest that demethylation of genes on the Xi may be a fairly common epigenetic mechanism in autoimmune disease. Several molecules thought to be of great potential importance for autoimmunity are encoded on the X chromosome; these include: the interleukin (IL) 2R γ chain (also known as the "common γ chain" because it is shared by receptors for IL2, IL7, IL15, and IL21);²¹² Foxp3, the master regulator in the development and function of Tregs;²¹³ tissue inhibitors of metalloproteinases 1–4, which are important in inflammation;²¹⁴ the X-linked inhibitor of apoptosis that regulates T cell function;²¹⁵ and toll-like receptor 7 and toll-like receptor 8, which are pattern-recognition receptors, both of which are important in recognition of single-stranded RNA.²¹⁶ There are also a large number of X-linked genes encoding possible target antigens of autoimmune disease: changes in dosage of these autoantigens could also provide an initiating event for development of autoimmunity. This is an area of research that is just opening up and it is likely that the next few years will dramatically increase our understanding of how X chromosome regulation might be involved in the development of autoimmunity.

Skewing of X inactivation, so that there is an overrepresentation of the Xa from one parent, has been reported in females with some autoimmune diseases, including autoimmune thyroid disease and scleroderma,^{217–220} but not in SLE or MS.^{221,222} As noted earlier, the process of X inactivation has multiple layers of epigenetic controls; thus, the potential for modulation of this process is high. Skewed X inactivation could influence expression of genes on the X chromosome and could lead to inactivation of a gene that protects against autoimmunity, or overexpression of a susceptibility gene, leading to increased autoimmune disease.

The next steps

Experimental evidence of a major role for epigenetically modified gene expression in the development of autoimmunity is accumulating at an ever-increasing pace, but there are many aspects still not understood. Over the last couple of years, the

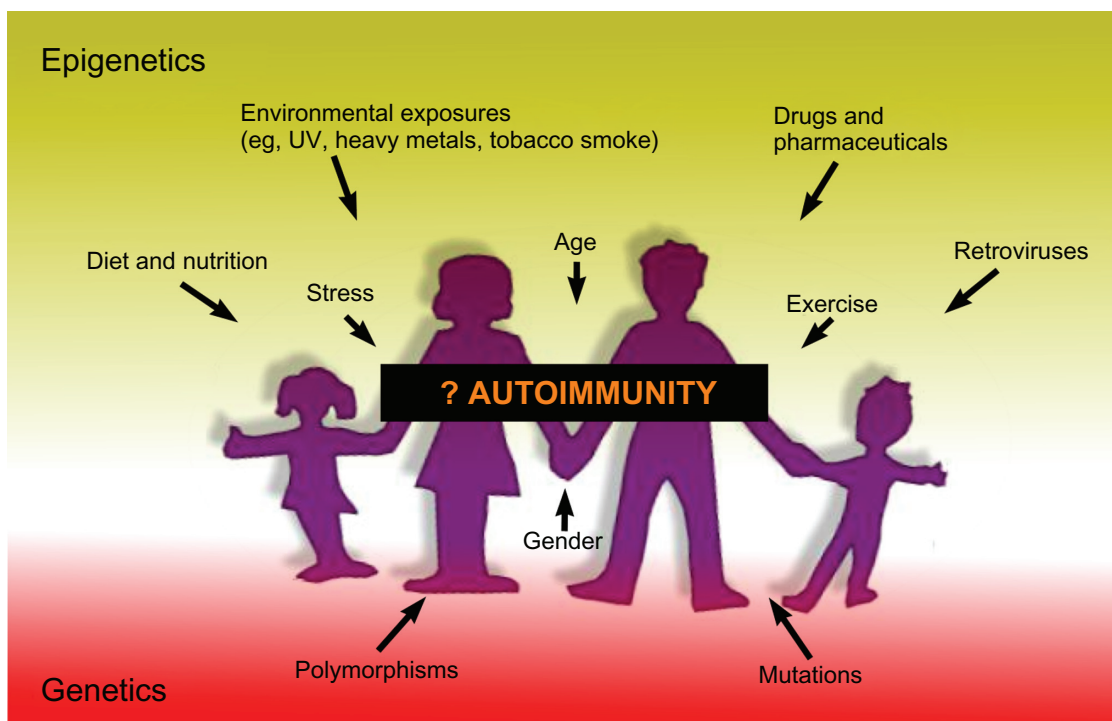


Figure 3 Summary of factors that may lead to the development of autoimmunity.

Notes: Underlying genetic factors, such as polymorphisms or mutations in certain genes, may be necessary to predispose to autoimmunity. Epigenetic factors, both from external sources (eg, environmental exposures, diet and nutrition, drugs and pharmaceuticals, retroviruses) and internal (eg, aging, gender, and stress and exercise levels), acting on the immune system and/or the target organ, may all contribute to either enhancing the development of autoimmunity or protecting against it. ? indicates that we are still unsure which of these factors might lead to autoimmunity.

Abbreviation: UV, ultraviolet.

global methylation measures, which have been used in many of the studies noted in this review, have been superseded by new technologies involving next-generation sequencing that can dramatically increase the amount and detail of the information that can be obtained regarding epigenetic modifications.^{223–226} Integrating the extensive genome-wide association study data that are available from many autoimmune diseases with methylome, chromatin epigenomic, and ncRNA data would be one way to potentially maximize our understanding of how the interaction of genetic and epigenetic factors increases susceptibility to or protection against autoimmune disease (Figure 3). Although, as has been pointed out,²²⁷ because epigenetic marks are known to change over the lifetime of an individual, it is harder to study with the same certainty as genetic association the association of epigenetics with disease.

However, improving our understanding of the role that epigenetic modifications play in the development of autoimmunity is likely to increase the prospects for controlling or preventing autoimmune disease through the use of drugs that target proteins controlling chromatin modifications (eg, HDAC inhibitors), DNA methylation (eg, inhibitors of DNA methyltransferases), or other epigenetic mechanisms.

Disclosure

The authors declare no conflicts of interest in this work.

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