

REVIEW

Epigenetics in Inflammatory Rheumatic Diseases

Lars C. Huber, Joanna Stanczyk, Astrid Jüngel, and Steffen Gay

Introduction

Inflammatory rheumatic disorders, such as rheumatoid arthritis (RA) and connective tissue diseases, are characterized by chronic inflammation that generally can be overcome by lifelong administration of immunosuppressive therapies. In most of these diseases, factors of genetic predisposition have been described, in particular the influence of distinct HLA haplotypes. In addition, environmental factors, including nutrition, infection, and exposure to sunlight, have been postulated as disease-driving agents. It is, however, unclear how genetic susceptibility in concert with the factors mentioned lead to the development of disease in one individual but not in another.

The successful accomplishment of the Human Genome Project, which has yielded sequences for ~25,000 identified human genes, presents a challenge to biomolecular research, since most of the identified genes code for biologic functions that have yet to be discovered. The functional characterization of these genes in normal physiologic processes as well as in the pathogenesis of diseases, a concept generally referred to as functional genomics (1), remains the main issue for biomedical research in the coming years.

Within the nucleus of a human cell, the DNA sequence contains ~3 billion basepairs, covering most of the nonheterochromatic portions of the genome. The low number of genes detected by the Human Genome Project is, however, most surprising. Moreover, between humans and chimpanzees, a remarkable genetic similarity of nearly 99% has been reported, thus highlighting the importance of distinguishing areas of the genome that would define genetic changes unique to humans (2).

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The low number of genes reflects the functional impact of posttranscriptional as well as posttranslational modifications and of epigenetic alterations in creating proteome diversity. Epigenetic alterations comprise heritable modifications of the DNA without any change in the base sequence of the genetic code per se. Two major groups of epigenetic alterations have been identified, i.e., DNA methylation and histone modifications. Changes to DNA or alternative splicing of messenger RNA (mRNA) transcripts has a strong impact on proteome complexity, and thus the utility of genomic information is somewhat restricted by the lack of precision in predicting genes, gene structures, and alternative splices (3,4). Epigenetic alterations can be inherited; however, changes can occur over the full span of a human life. When analyzing the epigenome (the genome-wide distribution of epigenetic alterations) in homozygous twins, Farga et al found a very similar epigenetic pattern in younger twin pairs, whereas older twin pairs showed several differences in the pattern of histone acetylation and DNA methylation, particularly in those whose lifestyles were not shared (5).

Currently, the epigenome is not measured systematically, and epigenetic changes have not been assessed within the Human Genome Project. For several illnesses, however, evidence indicates that they are linked to specific epigenetic processes and variations, which might provide an explanation for the late onset and strong age dependency of some diseases or the progressive nature of many common diseases (6). Moreover, it is likely that epigenetic alterations underpin the role of environmental factors in the pathogenesis of diseases in genetically susceptible individuals, and thus might broaden the scope of design of new therapeutic strategies. In this review article, we focus on the emerging concepts of epigenetics (summarized in Figure 1) as they apply to inflammatory rheumatic diseases.

Overview of epigenetics

Definition. The term epigenetics describes the broad array of biochemical processes that control the

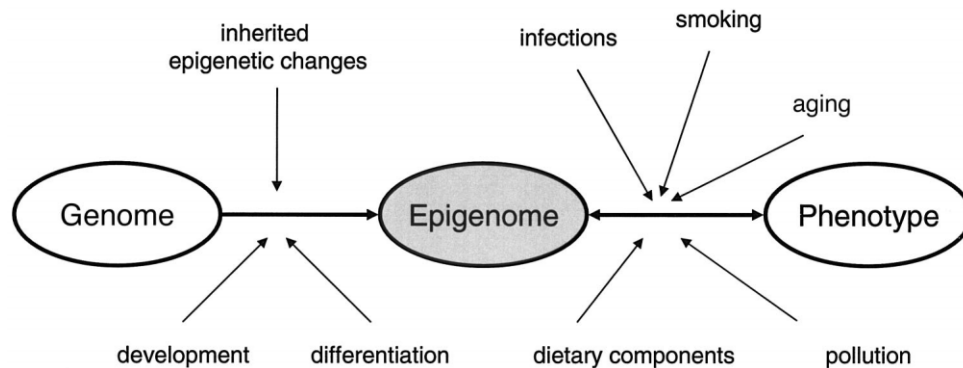


Figure 1. Genes, epigenetics, and phenotype. Postsynthesis modifications of DNA might be inherited or can become established during cell development and differentiation. Responses to environmental factors (including dietary components, aging, air pollution, smoking, and infection) affect epigenetic processes. Adapted from ref. 63, with permission from Elsevier.

functional state of the DNA but do not directly involve the DNA sequence of the A, G, T, and C nucleotides. This definition distinguishes epigenetic alterations from genetic mutations. Nonetheless, epigenetic changes are maintained during cell division and thus are heritable. Two major groups of changes define the epigenome of a cell: postsynthesis methylation of DNA, and modifications of histones that modulate the accessibility of transcription factors and promoters in presenting information on the DNA. In addition, epigenetic alterations are reversible, thus offering the opportunity to use pharmacologic approaches to reverse these changes and ameliorate the effects of a specific phenotype (7).

DNA methylation. The methylation of DNA is the only physiologic modification of postsynthesis DNA that will result in changes to its function (8). Whereas DNA methylation in prokaryotes may occur on cytosine and adenosine, methylation of DNA in eukaryotes is restricted to the pyrimidine base (cytosine). In its most common form, DNA methylation takes place at position 5 of the cytosine ring within CpG dinucleotides (Figure 2). Nonmethylated CpG dinucleotides are clustered in particular regions, called CpG islands. Generally, these islands surround the promoter region of protein-coding genes as well as housekeeping genes. Several DNA methyltransferases (Dnmt) that catalyze this methylating epigenetic modification have been identified. The biochemical details of DNA methylation, including folate metabolism, are provided in Figure 2.

The insertion of a methyl group into DNA leads to structural changes of chromatin and is associated with gene silencing. This repressive function, i.e., decreasing the level of gene expression, can be achieved by 2

principal mechanisms. 1) Structural modifications of cytosine prevent the proper docking of DNA-binding factors to their fitting DNA recognition sites. 2) Methyl-CpG-binding proteins can activate transcriptional corepressors, which leads to inhibition of gene expression (for details, see ref. 8).

Histone modifications. Genetic information is compactly organized within chromatin, a highly versatile protein-DNA complex that, once unravelled, can reach 2 meters in length in every nucleus. Nucleosomes comprise important substructures of chromatin and consist of an octamer of 4 core histones that is wrapped by 146 basepairs of DNA. This octameric histone structure is composed of an H3-H4 tetramer and 2 H2A-H2B dimers (9). An additional histone, histone H1, is involved in compacting nucleosomal DNA by linking both DNA and protein and plays a role in changing the path of the DNA as it exits from the nucleosome.

Apart from DNA methylation, local chromatin architecture, and thus transcriptional regulation, is strongly influenced by posttranscriptional modifications of histones, including methylation, phosphorylation, ubiquitination, sumoylation, and acetylation (10,11). In a highly compact state, the accessibility of DNA for basal transcription factors (e.g., the TATA box binding protein) and RNA polymerase II is limited, and the rate of gene expression is low (12). Conversely, modifications of histones lead to the local unwinding of DNA, and gene transcription is initiated.

Arguably, the best-characterized posttranslational modification of histones is the acetylation of core histones. Histone acetylation is catalyzed by histone acetyltransferases (HATs) and takes place at the

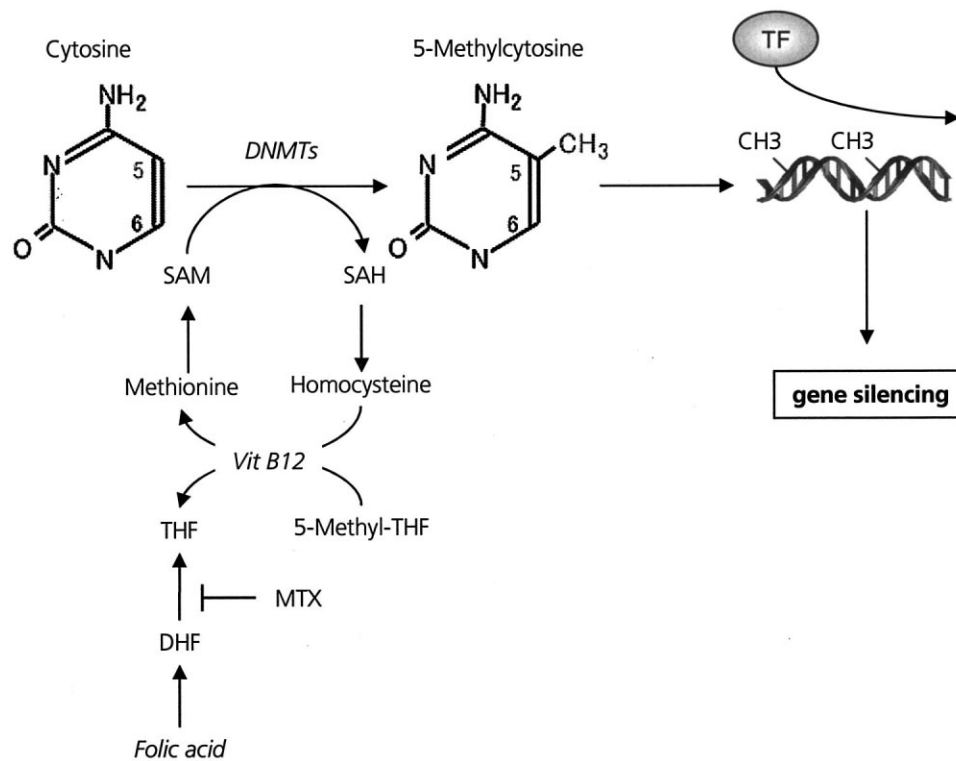


Figure 2. Mechanism of DNA methylation. DNA methyltransferases (Dnmt) use S-adenosylmethionine (SAM) as a donor of methyl (-CH₃), the generation of which is mainly modulated by nutritional folic acid. Briefly, folate is metabolized to dihydrofolate (DHF) and tetrahydrofolate (THF), and the latter step is inhibited by methotrexate (MTX). Vitamin (Vit) B₁₂ facilitates the generation of methyl-THF, which, in turn, catalyzes the conversion of homocysteine to methionine. Methionine is further metabolized by methionine adenosyl transferase to SAM, the substrate for Dnmt. Deficiencies of the enzymes involved in these processes result in hypomethylation of DNA and genomic instability. Several dietary components have been shown to enhance or silence gene expression selectively. For DNA methylation, folate and methionine are the major dietary sources of methyl groups, whereas vitamin B₁₂ is an important cofactor (63–65). The insertion of a methyl group into DNA leads to structural changes of chromatin, thus hindering transcription factors (TF) from binding, resulting in gene silencing.

ε amino groups of lysine residues at the N-termini. Hyperacetylation of histones is generally associated with enhanced rates of gene transcription. Regulation of gene transcription is thus modulated by the tight balance of histone acetylation and histone deacetylation. The targeted deacetylation of histones is performed by several multisubunit enzyme complexes, i.e., histone deacetylases (HDAs) (11,13) (Figure 3).

HDAs exert their function by removing the acetyl group from the nucleosomal core histones. The space between histones and surrounding DNA is reduced by hypoacetylation, and transcription factors are sterically hindered from binding, leading to gene silencing. The removal of an acetyl group is performed by a sophisti-

cated charge-relay system using Zn²⁺ ions as the prosthetic group. HDA inhibitors can inhibit the enzyme activity of HDAs. Trichostatin A (TSA), for example, fits into the active catalytic pocket of the enzyme and exchanges the zinc ion, leaving the enzyme dysfunctional (9,14).

In addition, both HATs and HDAs have a wide range of protein substrates beyond histones (13). These substrates can modify the activity of proteins involved in transcription, nuclear translocation, and cytoskeletal architecture. However, these issues are not within the scope of this review.

MicroRNA. MicroRNA are a class of short (19–22 nucleotides), noncoding RNA that induce silenc-

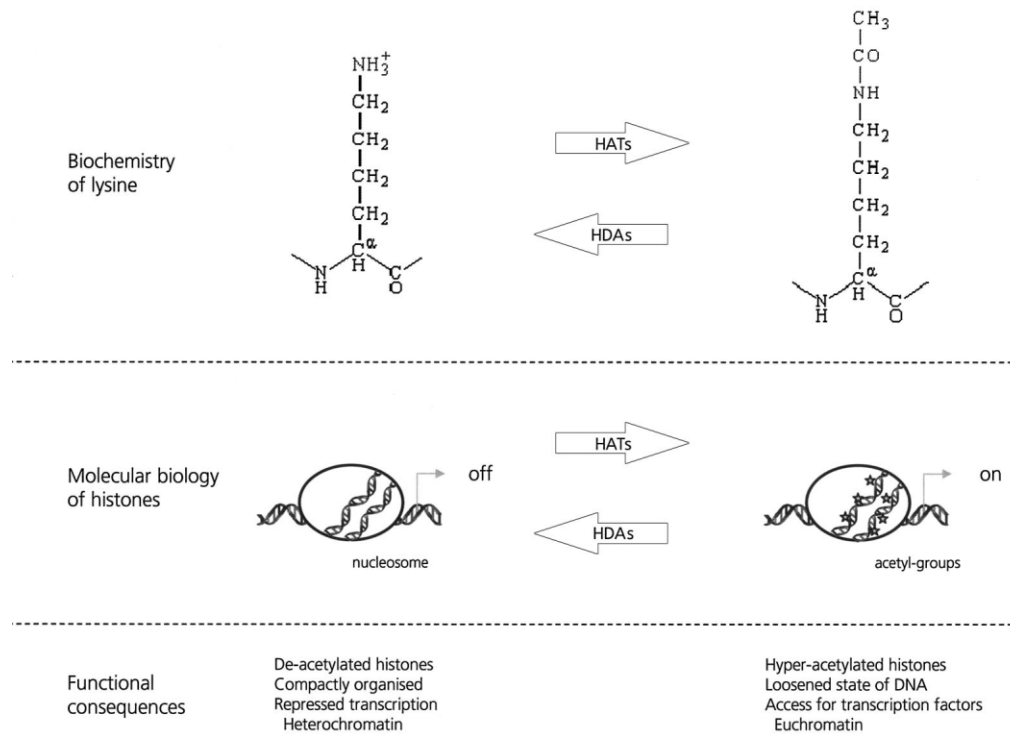


Figure 3. The tight balance of histone acetylation and deacetylation and its regulatory effects on the rate of gene transcription. **Top**, Histone acetylation is catalyzed by histone acetyltransferases (HATs) at the ϵ amino groups of lysine residues. Conversely, histone deacetylation is performed by histone deacetylases (HDAs), which remove the acetyl group from the nucleosomal core histones. **Middle**, The space between histones and surrounding DNA is reduced by hypoacetylation, and transcription factors are sterically hindered from binding, leading to gene silencing. **Bottom**, The removal of an acetyl group is performed by a sophisticated charge-relay system using Zn^{2+} ions as the prosthetic group. HDA inhibitors fitting in the catalytic site of HDAs are known as epigenetic inhibitors.

ing of protein-coding genes at the posttranscriptional level. MicroRNA have emerged as one of the most important and abundant regulators of gene expression (15). These tiny RNA molecules are generated from genome-encoded stem-loop precursors by the action of RNase III-type endonucleases known as drosha and dicer (16). The resulting short RNA duplexes, referred to as mature microRNA, recognize the 3'-untranslated region (3'-UTR) of target mRNA in a sequence-guided manner, and induce the degradation of the mRNA or repress protein translation (17).

A growing body of evidence has proven the involvement of microRNA in many biologic processes, including cellular differentiation, organ development, oncogenesis, metabolic processes, and viral infection (18–20). Interestingly, a role of dicer-generated RNA in immune regulation and autoimmunity has also been proposed (21). MicroRNA are genome-encoded, and most of them have been highly conserved during

evolution, suggesting an essential role of this RNA family in cellular biology (22). Some microRNA have a strikingly powerful biologic impact when misexpressed, as exemplified by the development of chronic lymphocytic leukemia caused by microR15 and microR16 gene deletion (23).

Moreover, accumulating data indicate the existence of functional links between epigenetic mechanisms and microRNA-mediated posttranscriptional control. In this regard, it has been suggested that altered methylation of microRNA genes might contribute to human tumorigenesis (24–26). HDA inhibitors, however, alter the expression of several microRNA in cancer cells (25,27), and microRNA have been associated with the expression of HDA-4 in developing rodent bones (28). These observations demonstrate the complexity of gene regulation within the transcriptome networks and highlight the close interplay between epigenetic and post-transcriptional mechanisms.

Boosted by the recent advances in technology, the field of microRNA research has enormously expanded in the last few years, thus providing high numbers of microRNA genes for further study and helping to elucidate the mechanisms of microRNA biogenesis and action. However, our understanding of microRNA function is still limited, and it is obvious that intensive research is necessary to obtain specific insight into the individual and global expression patterns of microRNA and how they contribute to cellular phenotypes in normal conditions and, even more importantly, in disease states.

Epigenetic processes in inflammatory rheumatic disorders

RA. RA is a chronic polyarticular disorder that mainly affects the synovial tissue of the joints. Progressive inflammation and the resulting erosion of the articular cartilage and adjacent subchondral bone cause severe pain, functional impairment, and, ultimately, disability (29). Like other autoimmune diseases, RA is a systemic disorder, and its exact etiology and pathogenesis are not yet fully understood. Nevertheless, it is generally accepted that autoimmune diseases emerge from a variable combination of individual genetic predisposition, environmental factors, and dysregulated immune responses (30,31). This complex interplay could be explained, at least in part, by the influence of epigenetic modifications.

In order to produce autoantibodies, mature B lymphocytes and plasma cells express CD21 (complement receptor 2) on their surface, and CD21 recognizes the complement component C3d present within immune complexes. Of interest, CD21 is expressed only by mature B cells but not by pro-, pre-, or plasma B lymphocytes, probably because mature B cells contain a methylated CpG island within the promoter region for CD21. In DNA derived from patients with RA, however, the CD21-expressing CpG island was found to be demethylated in peripheral blood mononuclear cells and synovial fluid mononuclear cells (32). These findings suggest a potential role of these cells in the dysregulation of the immune response in RA.

DNA methylation is also involved in the transcriptional regulation of endogenous retroviral sequences such as L1s (long interspersed nuclear elements). Human L1s contain a UTR and 2 open-reading frames (ORF1 and ORF2), the latter encoding for an endonuclease and a reverse transcriptase. Neidhardt and coworkers (33,34) showed that 3 of 5 CpG islands of the

genomic L1 5'-UTR were hypomethylated in RA synovial fibroblasts (RASFs). Genomic hypomethylation has been reported to be an inducer of LI elements, and therefore members of the stress-activated protein kinases, which are localized next to the L1 retrotransposon, might be activated.

With respect to histone acetylation, Ito and coworkers (35) showed that the activity of class I HDAs (in particular HDA-2) was strongly impaired in inflammatory airway diseases such as chronic obstructive pulmonary disease, asthma bronchiale, and adult respiratory distress syndrome. Reduced HDA activity leads to chronic hyperacetylation of histones, and thus to enhancement of transcription of genes encoding inflammatory proteins such as tumor necrosis factor α , interleukin-8 (IL-8), and matrix metalloproteinase 9. In our investigations, we similarly observed a reduction in total HDA activity in the synovial tissue of patients with RA, probably due to decreased tissue expression of HDA-1 and HDA-2 proteins, both of which were clearly detected at lower levels in RA synovial tissue as compared with osteoarthritis or normal synovium (36).

Ito et al further showed that HDA-2 suppressed NF- κ B-mediated gene expression (37). Previous studies indicated that NF- κ B is highly activated in RA synovial cells (for review, see ref. 38). Consistent with the findings of Ito and coworkers, we hypothesized that class I HDAs appear to act upstream of NF- κ B and other related transcription factors in RA (36). These findings suggest that reduced HDA activity plays a key role in the pathogenesis of inflammatory joint diseases.

It has also been shown that cell death could be induced, in a synergistic and dose-dependent manner, by cotreatment of RASFs with the HDA inhibitor TSA and TRAIL. In contrast, TRAIL alone and TSA alone had no effect or only a modest effect (39).

Systemic sclerosis (SSc). SSc (also known as scleroderma) is a rare, complex, multisystem disorder that is characterized by severe fibrosis of the skin and internal organs. As in other collagenoses, the etiology of SSc is unknown, and the exact mechanisms involved in the pathogenesis are not well understood. However, the following key pathogenetic processes have been identified: excessive accumulation of collagen and other components of the extracellular matrix, early morphologic changes in small blood vessels, and alterations in the cellular and humoral immune responses, resulting in the production of disease-specific antibodies (40). Currently, it remains to be elucidated how these processes interact to cause a chronic and progressive fibrotic disease. Nevertheless, epigenetic mechanisms have been

investigated to clarify the altered phenotype of the SSc fibroblast that leads to excessive deposition of extracellular matrix and reduced expression of matrix-degrading enzymes. These cellular alterations have been reported to be stable in multiple generations of SSc fibroblasts and might be transmitted by epigenetic imprinting. The persistence of this profibrotic phenotype thus might explain, at least in vitro, the development of clinical tissue fibrosis.

Wang and coworkers (41) explored the effects of the Dnmt inhibitor 2-deoxy-5-azacytidine (azaC) as well as the HDA inhibitor TSA on epigenetic modifications of SSc fibroblasts. The azaC inhibitor is a cytosine analog with a nitrogen atom at position 5 of the pyrimidine ring and is incorporated into newly synthesized DNA. The addition of these epigenetic suppressors normalized the expression of collagen in cell-cultured SSc fibroblasts. Moreover, in the promoter region of the collagen suppressor FLI1 (the oncogene known as Friend leukemia virus integration 1), DNA methylation was found within CpG islands, indicating the occurrence of gene silencing and overproduction of collagen in SSc (41).

The antifibrotic effect of TSA and other HDA inhibitors has been described repeatedly, and TSA was even postulated as a lead compound in the development of antifibrotic drugs (42–44). In this regard, we were able to show that the HDA inhibitor TSA had potent antifibrogenic effects on SSc skin fibroblasts in vitro (45). With respect to the pivotal role of profibrotic cytokines, including transforming growth factor β , platelet-derived growth factor, and IL-4, in the pathogenesis of fibrotic diseases, we found that TSA abrogates the stimulating effects of these factors on extracellular matrix production by preventing the cytokine-induced transcription of type I collagen α -1 chain (procollagen) and fibronectin (45).

With regard to the mechanisms of action of TSA, we observed up-regulation of the cell cycle inhibitor p21 and a decrease in the DNA-binding fractions of Smad3/4 complexes (45). Other investigators have not observed such effects (46), and it remains unclear how TSA blocks the translocation of these Smad molecules. Simonsson and coworkers (47) showed that the stability of the antifibrotic Smad7 was strongly reduced by HDA-1-mediated deacetylation, and, conversely, that the addition of TSA increased the half-life of Smad7 by preventing its ubiquitination. Thus, one could hypothesize that TSA reduces phosphorylation and nuclear translocation of activated Smad complexes by hyperacetylation of Smad7 (48).

Furthermore, in a common animal model of bleomycin-induced fibrosis, intraperitoneal administration of micromolar doses of TSA in mice prevented the dermal accumulation of dense collagen bundles and the replacement of subcutaneous fat tissue, as quantified by dermal thickness (45). Taken together, these findings emphasize the potential role of epigenetics in the pathogenesis of SSc.

Systemic lupus erythematosus (SLE). SLE (7) is an autoimmune disease that has the typical pathogenetic feature of production of autoantibodies directed against multiple nuclear antigens. The autoimmune response is thought to arise from defects in the apoptosis reactions and impaired removal of apoptotic material, leading to an overload of autoantigens that would normally hide from the immune system. The clearance, caused by dying cells, of autoantigenic material having autoimmune potential is a highly regulated process. Cells undergoing apoptosis are normally removed through non-inflammation-related engulfment by macrophages or even nonprofessional phagocytes. The clearance process involves cellular and humoral immune reactions to promote phagocytosis, before dying cells turn secondarily necrotic. An accumulation of such cells may occur when the apoptotic load exceeds the local capacity for phagocytes to mediate clearance, a situation that could reflect either increased cell death or failed clearance (49–52).

The most extensive evidence for a role of epigenetics in inflammatory rheumatic diseases has been obtained in patients with SLE, and multiple epigenetic factors have been postulated for the onset of this disease. T cells from SLE patients, for example, show aberrant changes in the pattern of DNA methylation (53), probably due to a decrease in the ERK signaling pathway, leading to low expression levels of Dnmt (54). Moreover, a decrease in Dnmt enzyme activities has also been proposed to occur in an age-dependent manner (55).

In a study by Richardson, it was noted that non-nucleoside inhibitors of DNA methylation (including the vasodilative drug hydralazine) cause lupus in susceptible individuals (56). Similarly, lupus-like disease in syngeneic mice was linked to demethylating agents (57), thus offering a potential explanatory model for the mechanisms of drug-induced lupus. Under the influence of demethylated DNA, T cells turn responsive to (auto)antigenic stimuli that are normally below the threshold of activation. Hypomethylated promoter regions of the cytotoxic molecule perforin might further contribute to the increased rate of apoptosis of monocytes and

macrophages, as reported recently (58). With respect to the production of autoantibodies, the B cell costimulating molecule CD70 was found to be overexpressed under the influence of demethylating agents, and this most likely led to an increased production of immunoglobulin (59).

Exciting new data helping to explain the strong predominance of SLE in women have been presented recently (60). CD40 ligand (CD154) is a B cell costimulatory molecule encoded on the X-chromosome and was found to be up-regulated on T cells in women with lupus. This overexpression might contribute to the production of autoantibodies. Since women have 2 X-chromosomes, one of which is inactivated by DNA methylation (as Barr body), the authors hypothesized that demethylation of CD40 ligand on the inactive X causes this unique overexpression of the B cell costimulatory molecule in women, rendering them more susceptible to the development of SLE. Similar processes of genetic imprinting might be involved in other autoimmune-related diseases.

Studies using HDA inhibitors have suggested that, apart from DNA methylation, histone deacetylation also might show abnormal patterns in SLE. In this regard, inhibition by TSA normalized the overproduction of IL-10 as well as the reduced production of interferon- γ in T helper cells derived from SLE patients (61).

Perspectives

Genetic analyses and studies of the potential role of environmental factors have been only partly successful in finding explanations for the complex pathogenesis of inflammatory rheumatic diseases. Epigenetic mechanisms, however, appear to play a pivotal role in modulating pathogenetic events in genetically predisposed individuals. Distinct from permanent genetic mutations, epigenetic alterations show an intrinsic plasticity and might well be targeted by pharmacologic strategies.

Several drugs that modulate the epigenetic reactions in rheumatic diseases have already been tested in vitro and in animal models. In particular, demethylating agents and HDA inhibitors might be of value therapeutically, but other targeted biologic agents that modify the epigenetic pattern will have to be designed. Such advances will increase the efficacy of these agents and minimize their toxic side effects.

To further elucidate the molecular pathways involved in the etiology and pathogenesis of inflammatory rheumatic disorders, epigenetic alterations have to be investigated and mapped along the lines of genome-wide

arrays (6). Therefore, international projects and organizations such as the DNA Methylation Society, the Epigenome Network of Excellence, and the Human Epigenome Project have been launched in recent years. Despite the huge financial investments required and the enormous complexities involved in the comprehension of the human epigenome (62), such projects will largely contribute to our understanding of the pathogenesis of diseases. Moreover, these insights will help to determine whether epigenetic drugs can be used clinically to treat or prevent disease on an individual basis.

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