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Mechanistic aspects of epigenetic dysregulation in SLE

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ABSTRACT

Epigenetic events have been linked with disease expression in individuals genetically predisposed to the development of systemic lupus erythematosus (SLE), a severe systemic autoimmune/inflammatory disease. Altered DNA methylation and hydroxymethylation as well as histone modifications mediate changes in chromatin accessibility and gene expression in immune cells from SLE patients. Defective epigenetic control contributes to uncontrolled expression of inflammatory mediators, including cytokines and co-receptors, resulting in systemic inflammation and tissue damage. While the pathophysiological involvement of epigenetic changes in SLE has been accepted for some time, we only recently started to investigate and understand molecular events contributing to epigenetic dysregulation. Here, epigenetic alterations will be discussed with a focus on underling molecular events that may be target of preventative measures or future treatment strategies.

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1. Introduction

Systemic lupus erythematosus (SLE) is a systemic autoimmune/inflammatory disease that is characterized by systemic inflammation, the presence of autoantibodies and autoreactive lymphocyte populations. Despite considerable efforts and recent advances in understanding the molecular pathophysiology of SLE, it remains largely unknown [1].

Mutations in single genes may result in severe immune dysregulation and the clinical picture of SLE or SLE-like disease. However, only 1–4% of all SLE patients develop so-called monogenic disease [1,2]. While sharing key clinical characteristics with "classical" SLE, monogenic disorders usually manifests early in life, show almost equal gender distribution. A considerable proportion of patients with "monogenic SLE" do not exhibit autoantibodies, or may develop them later as a secondary phenomenon. Indeed, most currently known monogenic forms of SLE or SLE-like disease fulfill criteria of autoinflammatory diseases, as opposed to autoimmune disease [2,3]. Monogenic disease and early-onset SLE are discussed elsewhere and are beyond the scope of this manuscript [4,5].

In most individuals with SLE, genetic predispositions increase the risk for disease, but additional factors are required for disease expression. "Pathophysiological co-factors" include female gender and associated hormonal factors (adolescent girls and women are 9–10 times more frequently affected than boys/men), environmental exposure (including infections, medication, toxins, chemicals, etc.), and epigenetic alterations that may be caused or influenced by the aforementioned factors. Usually, non-genetic factors accumulate over time, which may be the reason why 'classical' SLE usually manifests later during adolescence or early adulthood [1,2,5–8].

Over the recent 15–20 years, the role of epigenetic alterations in the molecular pathophysiology of SLE became increasingly clear [1,2,5–9]. Heritable, but generally reversible molecular events are summarized as epigenetic mechanisms. Under physiological conditions, epigenetic mechanisms control the accessibility of genomic DNA to components of the transcriptional complex, including transcription factors and RNA polymerases, regulating gene transcription in a cell-, tissue-, and signal-specific manner. Thus, epigenetic mechanisms are centrally involved in cell and tissue diversity in the human body (with the exception of gametes, all cells in the body share the same genotype) [10–12]. A number of molecular events contribute to shaping the epigenome, including DNA methylation, DNA hydroxymethylation, and histone modifications. Disturbances to the epigenome are involved in altered gene regulation, the expression of co-receptors, cytokines, and/or intracellular signals in SLE and other autoimmune disorders [2,12]. In the following, disease-associated epigenetic alterations and underlying molecular events will be discussed.

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2. Epigenetic alterations in SLE

2.1. DNA methylation

The addition of a methyl group to the 5′ carbon position of cytosine within cytosine-phosphate-guanosine (CpG) dinucleotides potently inhibits the recruitment of transcriptional regulators to genomic regulatory elements. DNA methylation is conferred by DNA methyltransferases (DNMTs). Maintenance DNMT enzymes (DNMT1 and DNMT2) are mainly responsible for re-methylation of daughter stands during cell division, while de novo DNMTs (DNMT3a and DNMT3b) confer DNA methylation at previously not methylated regions. However, this may be an oversimplification and some of the functions may be redundant between the two DNMT classes [2,6,7]. In addition to DNMTs, several proteins and pathways are involved in controlling DNA methylation patterns, including protein kinases and DNA repair pathways.

The central involvement of DNA methylation in the pathophysiology of SLE has been established. Global DNA methylation is reduced in lymphocytes from patients with SLE, and reduced DNA methylation correlates with disease activity [2,7,10]. However, DNA methylation patterns are complex, region-, cell-, and/or tissue-specific. Through interplay with other epigenetic events these are involved in molecular fine tuning [7,8]. The central involvement of DNA methylation in SLE pathology is particularly underscored by the observation of Javierre et al. that aberrant DNA methylation patterns distinguish between SLE patients and healthy siblings in genetically identical disease discordant monozygotic twins [13]. To date, a considerable number of genes in various immune cells has been demonstrated to undergo altered DNA methylation in SLE (Table 1).

2.2. DNA hydroxymethylation

Recently, DNA hydroxymethylation was identified as epigenetic mechanism and linked with the pathophysiology of autoimmune/inflammatory conditions, including SLE [58,59]. Hydroxymethylated DNA acts as an intermediate on the way from heavily methylated DNA to open chromatin through active and passive DNA demethylation (see below). Indeed, DNA hydroxymethylation is currently considered an activating epigenetic mark and reflects transcriptional activity of genes [60–62]. Indeed, the observation that depletion of hydroxymethylation conferring ten eleven translocation (TET) proteins does not necessarily result in increased DNA methylation, DNA hydroxymethylation is considered an independent and stable epigenetic mark that can exert by itself regulatory functions [63]. Generally, increased mRNA expression in active SLE patients suggests that increased TET activity may result in DNA hydroxymethylation and increased gene expression. Whether altered abundance or activity of TET proteins, or over-abundance of co-factors play a role in altered DNA hydroxymethylation in SLE remains elusive. Indeed, while on a genome-wide level increased, exact hydroxymethylation patterns are very complex and our understanding of its contribution to altered gene regulation in SLE is superficial [2,59,64,65] (Table 2) (Fig. 1).

2.3. Histone modifications

Post-translational modifications to N terminal amino acid restudies of histone proteins regulate accessibility of regulatory regions to transcription factors and RNA polymerases by adjusting their three-dimensional arrangement. In eukaryotic cells, histone proteins aggregate in octamers with two copies of each histone H2A, H2B, H3 and H4. Histone octamers form complexes with genomic DNA, and 147 base pair spanning stretches of DNA are wrapped around them. These DNA:histone complexes are referred to as nucleosomes [2,6,8,12]. Histone modifications are manifold and among others include acetylation, phosphorylation, citrullination, and methylation. Activating or "opening" histone modifications include H3 lysine 18 acetylation (H3K18ac)

or H3 lysine 4 tri-methylation (H3K4me3). Tri-methylation at Histone H3 lysine 9 (H3K9me3) or H3 lysine 27 (H3K27me3), however, result in chromatin condensation and epigenetic silencing.

Histone modifications and their cell-, tissue, and signal-specific patterns are complex. Together with DNA methylation patterns they determine the phenotype and function of cells and tissues. Over the recent years, it became clear that histone modifications contribute to the altered phenotype of immune cells and the pathophysiology of SLE [2]. Globally reduced histone H3 acetylation and H3K9 methylation are reduced in CD4+ T cells from SLE patients [67]. Most data on SLE-related histone modifications are available for cytokine genes (Table 3). CD4+ T cells from SLE patients exhibit permissive histone marks at the IL17 (H3K18ac ↑, H3K27me3 ↓) and the IL10 (H3K18ac ↑) gene clusters that allow for increased gene expression (16, 35). In contrast to these findings, the IL2 gene exhibits histone marks indicative for condensed chromatin (H3K18ac ↓, H3K27me3 ↑) which are reflected by failure to express IL-2 in T cells from SLE patients [53].

3. Molecular mechanisms orchestrating the epigenome in SLE

3.1. Regulation of DNA methylation

Over the recent years, a number of molecular mechanisms have been identified as contributors to altered DNA methylation patterns in SLE. Aforementioned DNMT enzymes are centrally involved in shaping the epigenome. Several studies provided evidence of altered DNMT expression in CD4+ T cells from SLE patients. However, results were not conclusive with both increased and reduced expression of DNMT1 and DNMT3 in cells from SLE patients [70–72]. However, seemingly inconsistent results may be explained by failure to measure protein expression levels and/or protein recruitment to regulatory regions. Furthermore, gene expression was not correlated with disease activity in individual patients.

DNMT-directed DNA methylation is tightly controlled and depends on signal-, target-, cell- and tissue-specific mechanisms, including protein kinase activity [36,44,45,73,74]. Reduced DNA methylation in T cells from SLE patients has been linked with altered MAPK activity. In patients with active disease, reduced activation of extracellular signal-regulated kinases (ERK) or increased activity of protein phosphatases, e.g. the serine/threonine phosphatase PP2A (that mediates ERK inactivation), results in impaired activation of DNMT1 and gradual DNA demethylation. Reduced activation of ERK protein kinases can furthermore be caused by impaired activity of protein kinase C δ (PKCδ) [73,74], a hallmark of T cells from SLE patients. Indeed, altered ERK activation and subsequent DNA demethylation through impaired DNMT1 activity has been linked to increased expression of co-stimulatory molecules CD11A [37,38,75], CD70 [76], CD40L [48,50], and the pro-inflammatory cytokine IL-17A [36,44,45], interferon-regulated genes [30], and the development of autoantibodies [7,24].

The DNA damage inducible protein GADD45α can mediate active DNA demethylation through complex interactions with activation-induced deaminase (AID), and the methyl-CpG-binding (MBD) protein MBD4. Further involving 5-methyl-cytosine-deaminase and G:T mismatch-specific thymine glycosylase, GADD45α can finally direct DNA demethylation. Since GADD45α expression is increased in T cells from patients with SLE, this mechanisms may contribute to global DNA demethylation in SLE [7,8,77,78]. The involvement of MBD4 in active DNA demethylation in SLE T cells was recently emphasized by Liao et al. who suggested reduced MBD4 expression in CD4+ T cells from SLE patients to contribute to active DNA demethylation of the CD70 promoter [79]. While it currently remains unclear why aforementioned increased MBD4 recruitment through GADD45α and AID, as well as reduced MBD4 expression in SLE T cells result in DNA demethylation at promoter regions and subsequently altered gene expression, both observations highlight a role of tightly controlled MBD4 expression and recruitment in active alteration of DNA methylation in SLE.

Table 1
DNA methylation patterns in SLE.

Reduced DNA methylation				
Gene	Cell type studied	Physiological function	Proposed effects in SLE	Ref.
CD6 (Cluster of differentiation 6)	T cells	T cell activation	T cell activation	[14]
CREM (cAMP response element modulator) P1 promoter	T cells, CD4+ T cells, effector CD4+T cells	Transcription factor	Generation of DN T cells; effector T cell differentiation	[15–20]
ESR1 (estrogen receptor 1)	PBMCs	nuclear estrogen receptor	Increased estrogen signaling; immune activation	[21,22]
Human endogenous retroviral elements (HERV)	CD4+ T cells, CD8+ T cells, B cells	No physiological function; remainders of ancient retroviral infections	Expression of interferon-related genes; auto-antibody production against HERV protein products	[23–26]
IFI44L (interferon-induced 44-like protein)	PBMCs	Unknown function; component of the type 1 interferon response	Immune activation	[27–29]
IKZF4 (IKAROS family zinc finger 4, encoding for Eos)	CD4+ T cells	Member of the IKAROS transcription factor family	Immune activation	[30]
IL4 (IL-4)	CD4+ T cells	Cytokine; involved in Th2 cell differentiation, maturation, and reduced apoptosis, B cell maturation, immunoglobulin class switch (IgE), etc.	Reduced lymphocyte apoptosis; B cell maturation; adhesion molecule expression	[31]
IL6 (IL-6)	CD4+ T cells	Cytokine; B cell proliferation, immunoglobulin production; hematopoiesis, thrombopoiesis; T cell proliferation, differentiation, cytotoxicity; acute phase response; etc.	B and T cell activation	[32]
IL10 (IL-10)	CD3+ T cells, CD4+ T cells	Inhibition of immune cells, antigen presentation and cytokine production	B cell activation, antibody production	[33–35]
IL13 (IL-13)	CD4+ T cells	Closely related to IL-4	Unclear, potentially as IL-4 (see above)	[33]
IL17A (IL-17A)	CD3+ T cells, CD4+ T cells, effector CD4+T cells	Induction of chemokines and cytokines; recruitment of neutrophils	Induction of tissue damage	[15,36]
IRF7 (Interferon regulatory factor 7)	CD4+ T cells	Activation of type I interferons	Immune activation	[30]
ITGAL (integrin alpha L; CD11A)	CD4+ T cells	Cellular adhesion and co-stimulation	T cell activation	[37–39]
KIR2DL4 (killer cell immunoglobulin-like receptor 2DL4; KIR)	CD4+ T cells	Detection of virus-infected cells	T cell activation	[40–42]
MX1 (myxovirus resistance 1 gene; interferon-induced GTP-binding protein Mx1)	Neutrophils	GTPase, mediates resistance against RNA viruses	Immune activation	[28]
PP2A (serine/threonine protein phosphatase 2A)	CD3+ T cells, CD4+ T cells	Protein phosphatase	Epigenetic remodeling of the IL17 gene cluster	[36,44,45]
PRF1 (Perforin)	CD4+ T cells	Cytolytic protein	Perforin expression in CD4+ T cells; T cell-induced death of monocytes/macrophages	[46,47]
TNFSF5 (tumor necrosis factor ligand superfamily member 5; CD40L/CD154)	CD4+ T cells	B cell co-stimulation	B cell co-stimulation; antibody production	[42,46,48–50]
TNFSF7 (tumor necrosis factor ligand superfamily member 7; CD70)	CD4+ T cells	B cell activation, IgG synthesis, T cell co-stimulation	B cell activation, IgG synthesis; T cell co-stimulation	[51]
Type I interferon response genes IFIT1, IFIT3, MX1, STAT1, IFI44L, USP18, TRIM22, BST2	Naïve CD4+ T cells	interferon-induced genes	DNA hypomethylation prior to T cell differentiation/activation suggests epigenetic poising that allows increased reactivity to type I interferon stimulation	[43]
Increased DNA methylation				
CD8A and CD8B (Cluster of differentiation 8A and B)	CD4+ T cells, CD8+ T cells, DN T cells	T cell surface co-receptor	Generation of effector CD3+CD4−CD8− T cells	[19,52]
IL2 (IL-2)	Bulk T cells, CD4+ T cells, effector CD4+T cells	Proliferation and activation of T cells	Reduced numbers and altered function of regulatory T cells; reduced activation-induced cell death; impaired function of CD8+ T cells; effector CD4+ T cell differentiation and cytokine expression	[8,20,53]
FOXP3 (Forkhead-Box-Protein P3)	PBMCs	Master regulator during the development and function of T_{reg}	Reduced numbers and altered function of regulatory T cells	[54–56]
NOTCH1 (Notch-1 trans-membrane receptor)	CD3+ T cells, CD4+ T cells	T cell lineage determination	T cell activation; IL-17A expression	[16]
NR3C1 (nuclear receptor subfamily 3 group C member 1; glucocorticoid receptor)	PBMCs	Regulates development, metabolisms, immune responses	Unknown; suspected increased immune activation	[57]

Table 2
DNA hydroxmethylation patterns in SLE.

Gene	Cell type studied	Physiological function	Proposed effects in SLE	Ref.
3826 genes with disturbed hydroxymethylation	PBMCs	Unknown	Immune dysregulation	[66]
CDKN1A (Cyclin-dependent kinase inhibitor 1A) Hydroxymethylation ↑	PBMCs	Regulation of G1 cell cycle progression, DNA replication and DNA damage repair; role during apoptosis	Defects in apoptosis and DNA repair	
CDKN1B (Cyclin-dependent kinase inhibitor 1B) Hydroxymethylation ↓	PBMCs	Regulation of G1 cell cycle progression	Defects in apoptosis or autophagy, resulting in increased exposure to nuclear autoantigens	
TREX1 (3′ exonuclease TREX1) Hydroxymethylation ↑	PBMCs	DNA repair; proof reading during DNA replication	Impaired exonuclease function and cytosolic DNA accumulation;	
		Association with the SET complex during granzyme A-mediated cell death	Reduced cell death and survival of autoreactive cells	
2748 genes with ↑ Hydroxymethylation	CD4$^+$ T cells	Unknown	Immune dysregulation	[64]
47 genes with ↓ DNA hydroxymethylation	CD4$^+$ T cells	Unknown	Immune dysregulation	
SOCS1 Hydroxymethylation ↑	CD4$^+$ T cells	Negative regulator of cytokine expression; role in T$_{reg}$ function	Immune dysregulation	
NR2F6 (V-erbA-related protein 2 (EAR-2)) Hydroxymethylation ↑	CD4$^+$ T cells	Nuclear orphan receptor, suppression of lymphocyte activation and Th17 responses	Autoreactive T cell activation	
IL15RA (IL-15 receptor alpha) Hydroxymethylation ↑	CD4$^+$ T cells	Promotion of T cell proliferation and activation	Autoreactive T cell proliferation and activation	

3.2. Regulation of DNA hydroxymethylation

Currently, our knowledge about DNA hydroxymethylation, its exact functions and underlying molecular mechanisms is very limited. Biochemically, DNA hydroxymethylation is the result of methylated cytosine oxidation within CpG dinucleotides by the ten eleven translocation (TET) family of methylhydroxytransferases [80–83]. To provide DNA hydroxymethylation, TET enzymes require co-factors, including Fe(II) and 2-oxoglutarate [80]. TET enzymes facilitate passive DNA demethylation and histone demethylation since hydroxymethylated DNA fails to bind DNMT1, which is required for DNA methylation maintenance, and methyl-CpG-binding (MBD) proteins that play a role in the translation of DNA methylation patterns into histone methylatio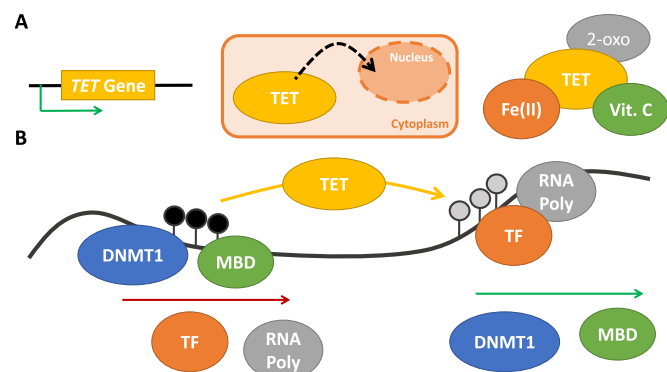n (see below) (Fig. 1) [84]. Furthermore, active DNA demethylation can be achieved though TET-mediated CpG DNA oxidation. The oxidation products 5-formylcytosine and 5-carboxylcytosine (by not 5-hydroxymethylcytosine) are excised by DNA repair enzymes and replaced with unmethylated cytosine [85].

3.3. Acquisition of histone modifications

A constantly growing number of enzymes have been reported to mediate specific epigenetic changes that allow fine-tuning of gene expression patterns. Posttranslational modifiers of N-terminal amino acid residues of histone proteins include lysine-specific histone acetyl-transferases (HATs), histone deacetylases (HDACs), lysine-specific methyltransferases (KMTs), and lysine demethylases (KDMs) [2,86].

Histone modifications cannot be understood and are not regulated as isolated events. They are mechanistically and functionally linked with DNA methylation and hydroxymethylation patterns. The family of methyl-CpG-binding (MBD) proteins play a role in the solidification of DNA methylation mediated transcriptional repression through the recruitment of additional epigenetic modifiers. Six MBD family members include MBD1 through MBD4, Kaiso, and methyl-CpG binding protein 2 (MeCP2), structural proteins that recruit histone deacetylases (HDACs) and others to genomic regions with high DNA methylation [2,87,88]. This contributes to the translation of DNA methylation patterns into silencing histone modifications. Thus, aforementioned reduced MBD4 expression in T cells from SLE patients likely not only mediate gradual DNA demethylation of the CD70 promoter, but also contribute to reduced solidification of DNA methylation patterns though the translation into histone modifications potentially across the genome [79].

Fig. 1. The role of TET proteins during DA hydroxymethylation. A) The presence and activity of TET proteins may be regulated by transcription of mRNA (left), altered protein stability or translocation to the nucleus (middle), or reduced or increased accessibility to co-factors, such as iron (Fe(II)), 2-oxoglutarate (2-oxo), or vitamin C (Vit. C) (right). B) TET enzymes facilitate DNA hydroxymethylation in an active and passive fashion. Active DNA demethylation can be achieved through CpG DNA oxidation. The oxidation products are excised by DNA repair enzymes and replaced with unmethylated cytosine. Furthermore, hydroxymethylated DNA fails to bind DNMT1 and methyl-CpG-binding (MBD) proteins that play a role in the translation of DNA methylation patterns into histone methylation (TF: transcription factor, RNA poly.: RNA polymerase).

3.4. Transcription factors orchestrate multi-dimensional epigenetic remodeling in SLE

The transcription regulatory factor cAMP response element modulator α (CREMα) is expressed at increased levels in T cells from patients with SLE. It centrally contributes to T cell dysfunction and resulting tissue damage [89,90]. CREMα belongs to the CREM superfamily of transcription factors that comprises more than 50 known isoforms [91]. Hormones and growth factors induce cAMP generation through

Table 3
Histone modifications in SLE.

Gene	Modification	Cell type studied	Physiological function	Proposed effects in SLE	Ref.
CD8A, *CD8B* (Cluster of differentiation 8A and 8B)	H3K18ac ↓, H3K27me3 ↑ during DN T cell generation	CD4$^+$ T cells, CD8$^+$ T cells, DN T cells	Lineage-defining surface co-receptor	Generation of CD3$^+$CD4$^-$CD8$^-$ DN T cells	[19,52]
ITGAL (integrin alpha L gene; CD11A)	H3K27me3 ↓	CD4$^+$ T cells	Cellular adhesion and co-stimulation	T cell-mediated inflammation	[68]
IL2 (IL-2)	H3K18ac ↓, H3K27me3 ↑	CD3$^+$ T cells, CD4$^+$ T cells, effector CD4$^+$T cells	Proliferation and activation of T cells	Regulatory T cells ↓; activation-induced cell death ↓ and longer survival of autoreactive T cells; function of cytotoxic CD8$^+$ T cells ↓; effector CD4$^+$ T cell differentiation and cytokine expression ↑	[20,53]
IL10 (IL-10)	H3K18ac ↑	CD3$^+$ T cells, CD4$^+$ T cells	Inhibition of T cell activation, B cell differentiation, activation, and immunoglobulin production	B cell activation, (auto-) antibody production	[35]
IL17A (IL-17A)	H3K18ac ↑	CD3$^+$ T cells, CD4$^+$ T cells, effector CD4$^+$T cells	Induction of chemokines and cytokines; recruitment of neutrophils	Tissue damage in SLE	[15,36]
TNF (Tumor necrosis factor alpha)	H3ac ↑	Monocytes	Monocyte activation, cytokine and prostaglandin production, priming of mononuclear cells, apoptosis, oxidative burst, induction of endothelial cell adhesion molecules and cytokine release, T cell apoptosis, etc.	Increased monocyte maturation and pro-inflammatory cytokine expression	[69]

adenylate cyclase, which in turn promotes the activation of protein kinases that activate CREM. Alternatively, T cell receptor complex (TCR) activation and calcium influx activate protein kinases, subsequently resulting in the activation of CREM family transcription factors [92,93]. In T cells, CREMα acts as transcriptional regulator with complex function. While it represses some genes, CREMα activates others. In CD4$^+$ T cells from SLE patients, CREMα contributes to the imbalanced expression of IL-2 and IL-17A [2,15,16,20,53,91,94–96]. It recruits to the *IL2* proximal promoter and mediates *trans*-repression and epigenetic remodeling through interactions with histone deacetylase HDAC1 and DNA methyltransferase DNMT3a [20,53,96] which mediate histone de-acetylation and DNA methylation. At the same time, CREMα orchestrates epigenetic 'opening' of the *IL17* gene cluster, comprising the pro-inflammatory homologues *IL17A* and *IL17F* [15,20]. CREMα recruits to the *IL17A* and *IL17F* proximal promoters in T cells from SLE patients [15,16]. Here, CREMα fails to recruit DNMT3a and HDAC1 while it actually induces DNA de-methylation and histone acetylation [15,16,20].

The exact mechanisms are currently unknown. However, this observation is in agreement with observations in male germ cells, where the CREM isoform CREMτ mediates histone acetylation [97]. The observation that CREMα interacts with the histone acetyltransferase p300 is of special interest in this context [96]. While CREMα recruits p300 to the *IL2* promoter, where p300 fails to be activated, the different transcription factor micro-environment at the *IL17* cluster may allow for p300 activation and histone acetylation [96,98]. Since p300 can physically and functionally link transcription factors, and since interactions with signal transducer and activator of transcription (Stat) family transcription factors mediate histone acetylation through p300 [35], a functional interaction between CREMα and Stat3 at *IL17A* appears likely (Fig. 2). Indeed, at the *IL10* gene, Stat 3 recruits to regulatory elements in the proximal promoter and the 4th intron where it co-recruits p300 resulting in histone acetylation and epigenetic 'opening' [35].

CD3$^+$TCR$^+$CD4$^-$CD8$^-$ "double negative" (DN) T cells exhibit effector phenotypes and are increased in number in the peripheral blood of
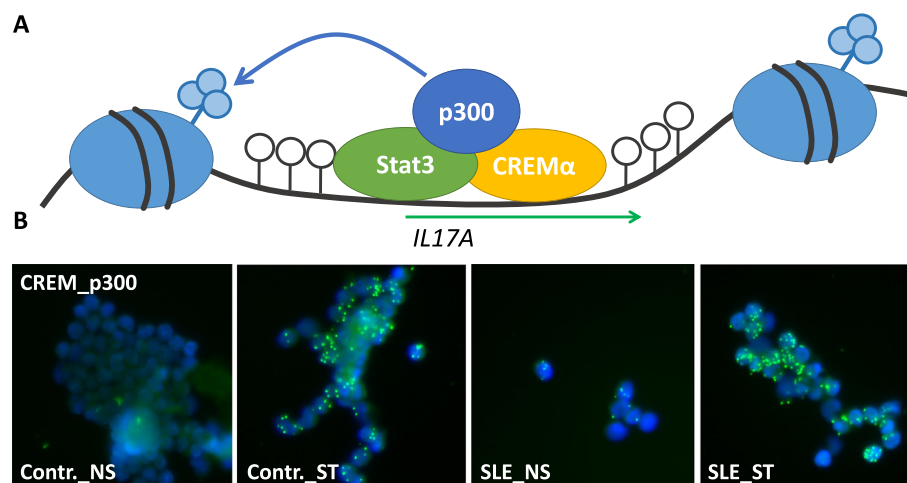

Fig. 2. CREMα instructs epigenetic 'opening' of the *IL17* cluster. A) The transcription factor CREMα recruits to regulatory elements along the *IL17* cluster. While the exact molecular mechanisms are currently unclear, CREMα instructs DNA demethylation (black open circles) and histone H3K18ac (blue circles). It appears likely that co-recruitment of the histone acetyltransferase p300 and co-localization with the transcription factor Stat3 may be involved. B) Representative images from proximity ligation assays (PLAs) indicating physical interactions between CREMα and p300. T cells from healthy controls (Contr.) and SLE patients were investigated under resting conditions (NS) or in response to stimulation with plate-bound anti-CD3 and anti-CD28 antibodies (ST). Nuclei are DAPI stained (blue), green signals indicate interactions between CREMα and p300. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

SLE patients. DN T cells infiltrate the kidneys during lupus nephritis, where they produce IL-17A and contribute to tissue damage [99,100]. We recently demonstrated that DN T cells can derive from CD8$^+$ T cells through the down-regulation of surface CD8 co-receptor expression (6, 18, 100). CREMα plays a central role in this process, since it recruits to conserved elements within the *CD8* cluster. Indeed, CREMα *trans*-represses the *CD8B* promoter and co-recruits DNMT3a and histone methyltransferase G9a to regulatory elements within the *CD8* cluster instructing epigenetic silencing and down-regulation of CD8A and CD8B expression (6, 18) (Fig. 3).

Of note, CREMα is not only directing epigenetic remodeling in effector T cells, it is also regulated on the epigenetic level. The *CREM* promoter P1 is responsible for the *trans*-regulation of CREMα. P1 is regulated by *trans*-activation through the transcription factor activating protein 1 (AP-1), which consists of the subunits c-Jun and c-Fos [101]. Transcription factor recruitment to P1 is regulated by DNA methylation and histone H3K4me3 [15–20,102] (Fig. 4A). In T cells from patients with SLE, reduced DNA methylation and increased H3K4me3 mediates epigenetic 'opening' of the promoter region and increased CREM transcription. Indeed, P1 H3K4me3, DNA methylation and CREMα mRNA expression reflect disease activity. While reduced DNA methylation and increased H3K4me3 allow transcription factor binding, including AP-1, Zhang et al. demonstrated that histone methyltransferase Set1 is responsible for the induction of H3K4me3 at P1. Since AP-1 co-recruits Set1 to other genes, it appears likely that this mechanisms may also be involved in the induction of epigenetic remodeling of *CREM* P1, since AP-1 recruitment to P1 is increased in SLE patients [101,103] (Fig. 4B).

3.5. Demographic and environmental factors affect the epigenome

Female gender is a strong contributor to the pathophysiology of SLE, and women are 9–10 times more frequently affected when compared to men. Estrogens affect T cell differentiation and subset distribution by the induction of epigenetic remodeling [104–107]. Indeed, estrogen receptor signaling enhances the expression of the transcriptional regulator CREMα, which exerts aforementioned strong effects on the epigenome in T cells favouring effector phenotypes [21,91].

In addition to estrogen levels, the presence of a second X chromosome in women contributes to increased SLE prevalence in women. A complex epigenetic event, referred to as X chromosome inactivation involves DNA methylation, histone modifications and non-coding RNA expression, and controls X chromosomal gene expression in women [2]. A number of X chromosomal genes contribute to the pathophysiology of SLE and other autoimmune disorders in a dose-specific manner [108]. Epigenetic disturbances at X chromosomal *TNFSF5* (CD40L) result in increased expression and immune dysregulation [50] (Table 1).

The "typical" gender distribution of adult-onset SLE is not present in the elderly. Indeed, elderly men exhibit even greater SLE incidences when compared to elderly women. With increasing age, epigenetic alterations accumulate and affect gene expression patterns [46]. A possible explanation may be reduced expression and activity of DNMT1 [51]. Subsequent gradual demethylation of genomic DNA may result
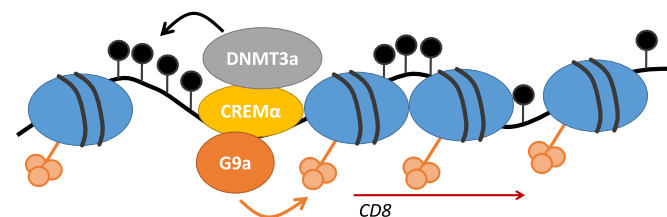
Fig. 3. CREMα instructs epigenetic "closing" of the *CD8* cluster. The transcription factor CREMα recruits to regulatory elements along the *CD8* cluster. At *CD8A* and *CD8B*, CREMα co-recruits the DNA methyltransferase DNMT3a and histone methyltransferase G9a instructing DNA methylation (black filled circles) and H3K27 tri-methylation (orange circles), resulting in DNA condensation and epigenetic silencing.
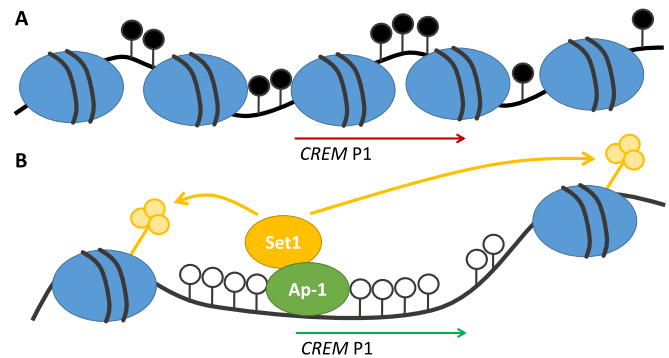
Fig. 4. The *CREM* promoter P1 is regulated by chromatin remodeling. Expression of the transcription factor CREMα is under the control of the *CREM* promoter P1. A) DNA methylation and inactivating histone marks silence CREMα expression. B) In effector T cells from SLE patients, DNA demethylation (black open circles) and Set1-mediated H3K4 tri-methylation (yellow circles) allow gene expression. CREMα expression is controlled by the transcription factor AP-1, which can co-recruit Set1 to regulatory regions. Though not experimentally proven for the *CREM* promoter P1, AP-1-directed recruitment of Set1 may contribute to increased CREMα expression in T cells from SLE patients. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

in the accumulation of so-called senescent T cells that are characterized by reduced expression of the surface co-receptor CD28, reduced telomere length, and increased expression of lupus-associated "inflammatory genes" [31,41,109].

Several environmental and chemical exposures can induce epigenetic remodeling and contribute to autoimmune phenomena. Hydralazine, a therapeutic agent used to treat hypertension, inhibits the protein kinase PKCδ. As mentioned above, reduced activity of PKCδ results in impaired ERK activation and altered activity of DNMT1 [73,74]. Methionine adenosyl transferase (MAT) is a redox-sensitive enzyme in the S-adenosyl methionine (SAM) cycle. In the presence of adenosine triphosphate (ATP), MAT converts methionine into SAM [110]. Thus, the availability of methionine may directly affect SAM generation. Since global DNA methylation is reduced in the elderly and may in some individuals contribute to autoimmune phenomena, alterations in the SAM cycle, and/or reduced dietary intake of methionine together with reduced DNMT1 activity may be central mechanisms [111].

As mentioned above, increased activity of GADD45α can result in gradual DNA demethylation in SLE patients. This is also of interest in the context of environmental exposure, since GADD45α is induced by UV irradiation, which is also a known trigger of disease flares in SLE and other autoimmune disorders [59,78,112].

4. Epigenetic events as therapeutic targets

As mentioned above, epigenetic alterations in autoimmune disease can be caused or affected by behaviour and environmental exposure. Thus, reduction of exposure to certain chemicals, UV light, etc. is already included in recommendation for patients.

In contrast to cancer therapy, "epigenetic treatment" is not currently available for SLE. However, several already available treatment options affect the epigenome and may correct alterations in SLE patients. Methotrexate, which is used in SLE patients with arthritis or skin involvement, can reduce the activity of DNMT1 by depleting SAM [113,114] and thereby affect regionally increased DNA methylation (e.g. *CD8*, *IL2*). Mitochondria are involved in oxidative stress. Activation of the enzyme mechanistic target of rapamycin (mTOR), they regulate DNA methylation through the inhibition of DNMT1 [73]. Antioxidants are already available, and indeed can reduce oxidative stress and mTOR activity, as shown for *N*-acetylcysteine [115–117]. Cyclophosphamide is used in individuals with severe SLE. It increases DNA methylation through induction of DNMT1 activity [51] and may therefore correct the expression of genes with reduce DNA methylation (e.g. *IL17*, *IL10*, etc.).

However, all of these effects are not targeted and can cause severe side-effects.

To date, no targeted approaches are available to correct DNA hydroxymethylation patterns. However, data indicates reduction of DNA hydroxymethylation in RA patients in response to methotrexate treatment [59,118]. Studies in cancer promise potential for indirect TET inhibition. The IDH1 inhibitors AGI-5198 and HMS-101 reduce DNA hydroxymethylation in tumor cell lines, which may be translatable to autoimmune/inflammatory disease [119,120].

Histone modifications have not directly been targeted in SLE treatment yet. However, several available treatment alter the histone code. Mycophenolate mofetil influences histone marks while not changing DNA methylation [121]. Inhibitors of the histone methyltransferase G9a (e.g. tramezostat) are in the pre-clinical phase of cancer studies [122]. Several available drugs act as inhibitors of HDACs, including suberoylanilide hydroxamic acid (SAHA) and trichostatin A (TSA), which both ameliorate disease in lupus-prone mice [123,124]. However, treatment with valproic acid (that also inhibits HDACs) can cause SLE-like disease in patients with epilepsy [125].

Taken together, preliminary observations indicate effects of already available treatment options and "new" treatments on chromatin conformation and gene expression. However, effects are not targeted and lack region or gene specificity. Thus, beneficial effects at one or several genes may coincide with dysregulation of previously unaffected genes. Targeted approaches aiming at underlying molecular alterations (e.g. transcription factor expression, etc.) may allow individualized correction of epigenetic alterations in the future.

5. Conclusions

The identification of epigenetic alterations as contributors to SLE has improved our understanding of disease pathology. Linking epigenetic patterns with contributing molecular events has further improved our understanding and delivered explanations for (at least some) demographic and environmental contributors. These advances promise potential for future applications in disease prevention and/or individually tailored and target-directed therapeutic approaches. However, additional studies are needed to fill in the blanks and allow the application of epigenetic patterns as disease biomarkers and/or therapeutic targets. To achieve this, cautiously planned collaborative approaches are necessary to link prospectively collected and meaningful clinical datasets with associated genetic and epigenetic patterns, underlying molecular contributors, and disease outcomes.

Acknowledgements

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