REVIEW

Epigenetics: An opportunity to shape innate and adaptive immune responses

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Abstract

Epigenetics connects genetic and environmental factors: it includes DNA methylation, histone post-translational modifications and the regulation of chromatin accessibility by non-coding RNAs, all of which control constitutive or inducible gene transcription. This plays a key role in harnessing the transcriptional programs of both innate and adaptive immune cells due to its plasticity and environmental-driven nature, piloting myeloid and lymphoid cell fate decisions with no change in their genomic sequence. In particular, epigenetic marks at the site of lineage-specific transcription factors and maintenance of cell type-specific epigenetic modifications, referred to as 'epigenetic memory', dictate cell differentiation, cytokine production and functional capacity following repeated antigenic exposure in memory T cells. Moreover, metabolic and epigenetic reprogramming occurring during a primary innate immune response leads to enhanced responses to secondary challenges, a phenomenon known as 'trained immunity'. Here, we

discuss how stable and dynamic epigenetic states control immune cell identity and plasticity in physiological and pathological conditions. Dissecting the regulatory circuits of cell fate determination and maintenance is of paramount importance for understanding the delicate balance between immune cell activation and tolerance, in healthy conditions and in autoimmune diseases.

KEYWORDS

adaptive immunity, autoimmunity, epidrugs, epigenetics, Foxp3, innate immunity, T-cell differentiation, Treg cell

INTRODUCTION

The definition of epigenetics includes reversible processes which affect gene expression—without DNA sequence changes—and can be inherited through cell generation, contributing to the maintenance of cell phenotype [1, 2].

Epigenetic modifications include DNA methylation and histone changes (post-translational methylation and acetylation) which regulate gene expression by modulating chromatin conformation and accessibility. DNA methylation, associated with gene silencing, is catalyzed by DNA methyltransferases (DNMTs) on cytosines located at CpG islands, in close proximity to promoter or distal *cis*-regulatory enhancer elements [3]. Different epigenetic enzymes, such as histone methyltransferases (HMTs) and acetylases (HATs), catalyze the apposition of the post-translational modifications—hence defined epigenetic writers—distinct from those responsible for histone demethylation (HDM) and deacetylation (HDAC), referred to as epigenetic erasers. While histone acetylation associates with a permissive chromatin state, methylation can be either favourable or not, depending on the number and the position of the methyl groups on the histone tail. Heterochromatin protein (HP)1 recognizes trimethylated H3 lysine (K)9 or K27 (H3K9me3 or H3K27me3, respectively) and induces chromatin silencing; on the contrary, nucleosome remodelling factor (NURF) identifies the histone mark H3K4me3 that associates with a permissive chromatin state [4]. The overall combination of histone modifications, defined 'histone code', designates chromatin accessibility to transcription factors (TFs). However, how chromatin conformations manage differentiation and lineage commitment from haematopoietic stem/progenitor cells (HSPCs) to mature immune cells has not been fully understood. Intriguingly, despite the intrinsically repressive state of the chromatin, lineage-promoting TFs can reach some of their binding sites even when they are wrapped into nucleosomes, recruiting chromatin-remodelling enzymes and exposing the underlying DNA. While certain TFs induce lineage-specific chromatin accessibility, others can play key roles in cell reprogramming [5, 6]. In immune cells, a network of regulatory elements (REs) and TFs coordinate transcriptional and phenotypic diversity. During development, inaccessible REs are recognized by pioneer TFs in a sequence-specific manner, leading to chromatin remodelling, which spreads heritable epigenetic information instructing cell identity. Recent studies have identified cell type-specific super-enhancers (SEs), defined as genomic regions which positively regulate the expression of genes that drive cell identity and lineage specificity; they designate complex REs distinct by high density of TFs and enhancer marks, common to cell lineage- and disease-associated genes [7]. Both intrinsic and extrinsic signals recruit TFs and transcriptional co-activators to SEs; along with the formation of chromatin multi-loop hubs, the result is that REs and their target genes are brought into close proximity. However, how intrinsic and extrinsic cues converge on enhancer activities to coordinate cell type- or transitory-gene expression profiles in immune cells is still not well understood. In this work, we discuss current advances on the epigenetic and transcriptional regulatory circuits that promote and restrict immune cell identity and function. We also propose possible future directions of investigation aimed at taking advantage of these mechanisms to control immune cell function in health and autoimmunity.

Epigenetic regulation as a bridge between the genome and the environment

One of the most relevant aspects of epigenetics is that it operates as a bridge between genotype and life experience. Both cell-specific- and environment-related changes in gene expression patterns can be determined by inheritable but reversible modifications of the DNA, histones and chromatin conformations together with non-coding RNA [8] (Figure 1). This research field is significantly improving our understanding of how the environment shapes our own phenotype along with the phenotype of our descendants, being potentially transmitted to the following generation.

F I G U R E 1 Epigenetic mechanisms (Histone modifications, DNA Methylation and Non- coding RNAs) regulate immune cell growth, development and function thanks to their ability to regulate gene transcription and genomic stability. Ac, acetyl, HAT, histone acetyltransferase; HDAC, histone deacetylase; HDM, histone demethylase; HMT, histone methyltransferase; Me, methyl. Figures have been created with BioRender.com.
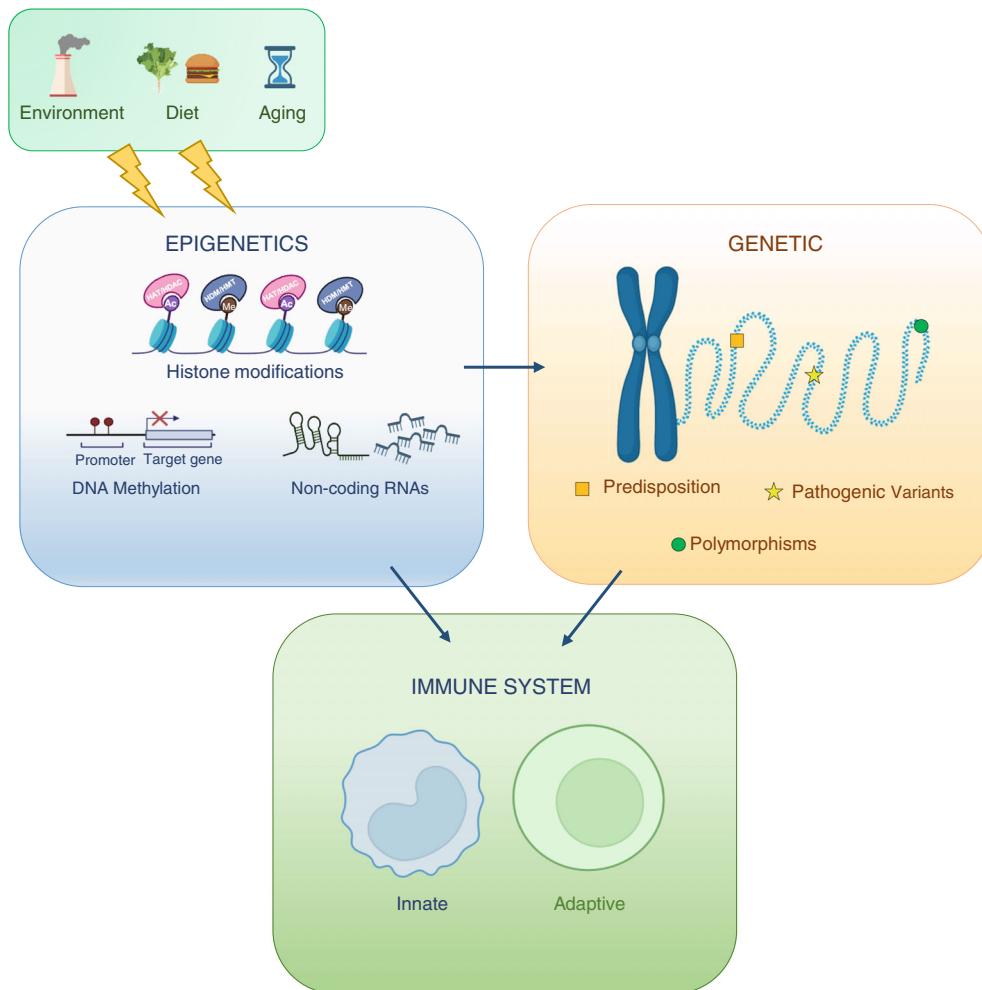
Interestingly, some environmental factors are so robust that even monozygotic twins can be distinguished through the analysis of their epigenetic traits [9]. Futhermore, the cell-specific gene expression patterns can be altered by the environment throughout the life, leading to phenotypical changes that may either protect or predispose to several diseases [1].

Certain dietary components can change gene expression via alterations in DNA methylation and histone modifications. Indeed, DNA and histones are modified by writers and erasers, whose activity is regulated by metabolic intermediates [10]. Nutritional composition and maternal diet contribute to the establishment of the epigenetic profile in the foetus that may affect the individual susceptibility to certain diseases, resulting in potential long-term consequences in the offspring [11]. Among the epigenetic modifications, a reduction in DNA methylation was found on the promoter of the insulin-like growth factor 2 (*IGF2*) gene (important for the modulation of foetal development and growth) in famine-exposed offspring compared to sex-matched controls [12, 13]. In addition, early exposure to famine caused low birth weight, cardiovascular diseases and low lipoprotein levels in the offspring [14, 15]. Moreover, maternal malnutrition has been associated with the development of metabolic diseases in adult offspring. Jousse et al. found a loss of methyl groups in the promoter of the *leptin* gene in adipocytes, corresponding to reduced levels of leptin mRNA in murine male offspring from mothers exposed to a low-protein diet during gestation and lactation [16]. Low-protein diet-induced hypomethylation of glucose-6-phosphatase (*G6PC*) promoter in piglet male offspring, together with increased methylation of H3K4 in the *G6PC* promoter in the liver. This led to the activation of the *G6PC* gene in males and an increased susceptibility to develop hyperglycaemia and diabetes in adulthood [17]. During pregnancy, maternal obesity increases the risk of obesity and metabolic diseases in the progeny. It has been shown that the offspring of mothers fed a high-fat diet showed hypermethylation in genes implicated in liver fibrosis and lipid accumulation, such as ephrin type-B receptor 2 (*Ephb2*) and fibroblast growth factor 21 (*Fgf21*) that could predispose to the development of fatty liver disease in the progeny [18].

Furthermore, epigenetic alterations that change the chromatin accessibility, resulting in abnormal gene transcription and/or genomic instability, have been proposed as key regulators of the ageing process, driver of age-related diseases (Figure 1). With age, the immunocompetence becomes constrained [19] and this associates with the repression of genes controlling immune cell differentiation along with the hyper-activation of autoimmunity/inflammation-related genes [20]. In macrophages, epigenetic mechanisms contribute to the reduced expression of the major histocompatibility complex (MHC)-II observed with age; epigenetic alterations also contribute to the low-grade inflammation associated with resting neutrophils due to increased levels of tumour necrosis factor (TNF)-α and interleukin (IL)-1α [20]. Dozmorov et al. reported hypomethylated regions showing T-cell-specific enrichment in active enhancers marked with H3K27Ac and H3K4me1 in elderly individuals, suggesting a progressive age-associated shift toward a pro-inflammatory phenotype that could contribute to the increased frequency of autoimmunity with age [21]. Interestingly, disease-associated genetic variations often occur within the SE regions of disease-relevant pathogenic cells [22]. Notably, single nucleotide polymorphisms (SNPs) predisposing individuals to autoimmune disorders are clustered in genomic regions with epigenetic modifications of active enhancers in T or B lymphocytes. More in detail, among the 76 SNPs linked to type 1 diabetes (T1D), 67 appear in non-coding sequences, with 13 occurring in the SEs of T-helper (Th) cell-specific genes. Likewise, in human subjects with systemic lupus erythematosus (SLE), the non-coding SNPs occur most frequently in B-cell super-enhancers, with 22 SNPs in the SEs of genes controlling B-cell maturation and function [22]. Furthermore, the analysis of exhausted CD8$^+$ T cells in humans and in mouse model of chronic viral infection has revealed distinct chromatin accessibility compared to memory CD8$^+$ T cells. This suggests that CD8$^+$ T cell exhaustion is supported by a broad remodelling of the enhancer and TF binding landscape, which features their distinct differentiation state [23]. Considering that both intrinsic and external factors modify epigenetic marks throughout life, a major effort should be dedicated to clarifying the relation among epigenetics, immune cell function and immune-related disorders.

Epigenetic control of innate immune cell function

The epigenetic scenario of innate immune cell regulation is quite complex. Innate cells work rapidly through the activation of short-lived transcription programs that are dependent on dynamic chromatin states [24]. The epigenetic regulation is crucial for the reprogramming of macrophages, governing the M1/M2 phenotypes [25–27] through histone modifications [26, 28, 29]. The H3K4- and H3K36-specific methyltransferase SET and MYND domain-containing 2 (Smyd2) suppresses *Il-6* and *Tnf* transcription and MHC-II expression and abolishes nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and extracellular signal-regulated kinase (ERK) signalling. Moreover, macrophages expressing high levels of Smyd2 impair Th17 but support regulatory T (Treg) cell differentiation, leading to TGF-β increase and IL-6 decrease [30]. M1 polarization is induced by the silencing of the *SOCS1* gene. Over-expression of SOCS1 and ten-eleven translocation methylcytosine dioxygenases (TET)2/TET3 and down-regulation of DNMT1 promote LPS- and IFN-γ-induced M1 activation [31–33] (Figure 2a). SIRT1 and SIRT2, cooperating with DNMT3b, are activated by macrophage differentiation and suppressed by the up-regulation of inflammation-related genes [34]. In lung and pancreatic cancer, HDAC inhibition modulates the production of nitric oxide (NO) in tumour associated macrophages (TAMs), leading to an anti-tumour effect [35]. Indeed, HDAC inhibitors modulate TAM phenotype and reduce the tumour burden in a murine model of breast cancer [36]. Despite accumulating evidences on the role of histone modifications in macrophage polarization, their specific role in TAM activation needs further exploration. The involvement of DNA methylation in modulating TAM phenotype is also still poorly characterized. Moreover, epigenetic mechanisms regulate macrophage-dependent tolerance during the exposure to the intestinal microbiota. Indeed, short-term stimulation of the pattern recognition receptor (PRR) NOD2 results in increased H3 and H4 acetylation on the promoters of cytokine-related genes in macrophages [37]. However, during prolonged NOD2 stimulation, both the acetylation and cytokine secretion dramatically decrease. Chronic NOD2 stimulation leads to up-regulation of Twist1 and Twist2 which bind to the *HDAC1* and *HDAC3* promoters driving their expression. HDAC1 and HDAC3 then mediate histone deacetylation at cytokine-gene promoters and, in turn, down-regulation of their expression. A similar regulatory loop was also reported upon chronic stimulation of multiple PRRs [37]. Another important aspect of epigenetics in profiling macrophage biological functions is the ability of local environment to shape their own identity [38]. Lavin et al. demonstrated that tissue-resident macrophages have distinct enhancer landscapes. More in detail, the enhancers of the gene encoding the transcription factor spalt-like transcription factor 1 (*Sall1*) are active (H3K4me1 and H3K27ac) only in microglia, while the
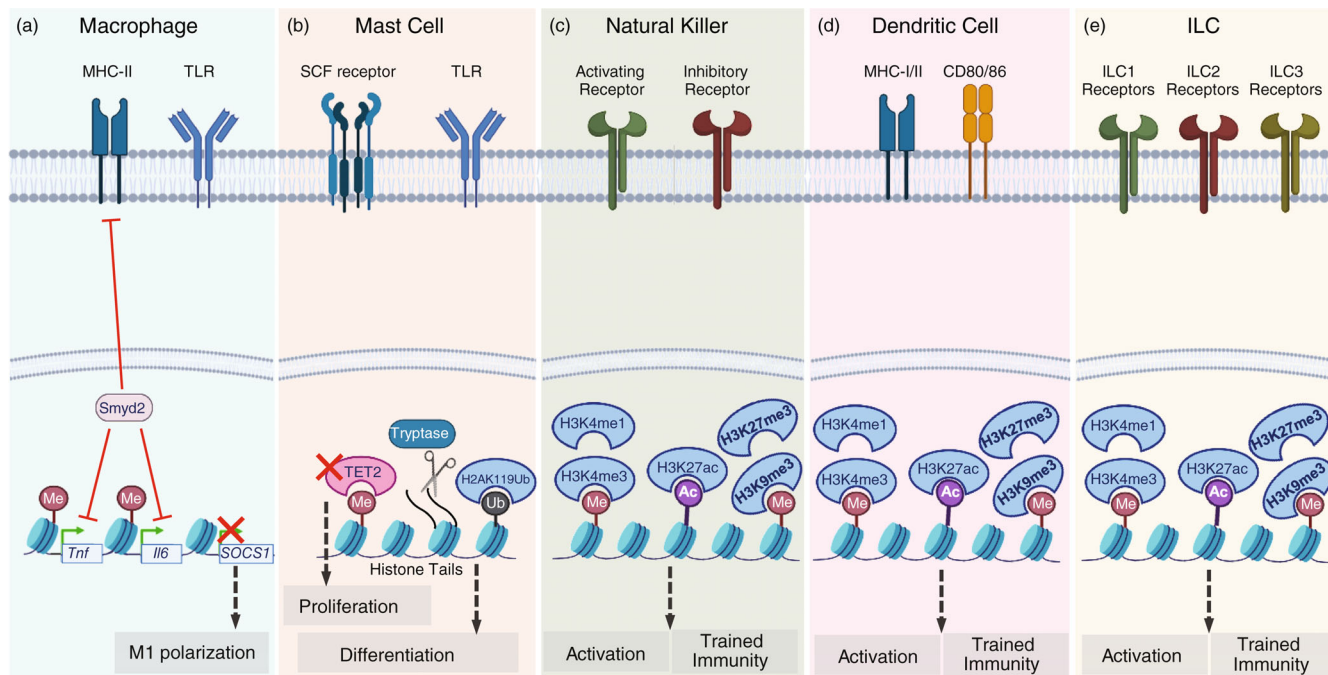
FIGURE 2 Epigenetic modifications in innate immune cells. Key epigenetic mechanisms (such as methylation/acetylation of DNA and histones) occurring in innate immune cells are implicated in proliferation, differentiation and trained immunity (a–e). Ac, acetyl; ILC, innate lymphoid cells; Me, methyl; MHC, major histocompatibility complex; SCF receptor, stem cell factor receptor; Smyd2, SET and MYND domain containing 2; SOCS1, suppressor of cytokine signalling 1; TET2, Tet methylcytosine dioxygenase 2; TLR, toll-like receptor; TNF, tumour necrosis factor; Ub, Ubiquitin. Figures have been created with BioRender.com.

enhancers of GATA Binding Protein 6 (*Gata6*) gene only in peritoneal macrophages [39]. Intriguingly, PU.1, the macrophage lineage-determining TF, can act with other TFs to organize the chromatin in a cell-type-specific manner and is required for the deposition of H3K4me1 at macrophage-specific enhancers [40, 41].

Mast cells are tissue-resident immune cells, playing a key role in allergic disorders and cancer [42, 43]. A hallmark feature of mast cells is their high content of cytoplasmic secretory granules packed with specific proteases including tryptase, chymase and carboxypeptidase A3 [44]. It has been shown that during apoptotic mast cell death, tryptase migrates from the granule compartment in the cytoplasm to the nucleus. H2A, H3.1 and, to a lesser extent, H4 are cleaved in their N-terminus into small fragments, γ-tryptase [45](Figure 2b). Histone modifications, such as ubiquitination of lysine 119 on histone H2A (H2AK119Ub), affect mast cell biology and promote their differentiation [46]. DNA methylation-related processes modulate mast cell proliferation and function [47]. Indeed, mast cells lacking *TET2* proliferate more than wild-type cells suggesting that *TET2* mutations may predispose to excessive mast cell proliferation [48] (Figure 2b). Interestingly, TET2 mutations affect at least 20% of patients with mastocytosis, a clonal proliferative disorder of mast cells, and correlate with worse overall survival [49, 50]. By using mast cell lacking DNA

methyltransferase enzyme DNMT3A, Leoni et al. demonstrated that this enzyme restrains mast cell response to several stimuli, both in vitro and in vivo [51, 52]. As the efficiency of the innate response strictly relies on cell differentiation, proliferation and activation, epigenetic regulation allows innate cells to modify their phenotype and resolve the damage. As double edge sword, these mechanisms also contribute to innate cell-related disorders since aberrant pathways could sustain disease-associated phenotypes.

Transcriptional memory and trained immunity

Immunological memory has been traditionally associated with the adaptive system but also innate immune cells can become more protective against infections after encountering pathogens or live attenuated vaccines, a memory phenotype named 'trained immunity' [53]. This phenomenon is based on two principal mechanisms: the epigenetic and metabolic reprogramming of innate immune cells. The priming event of trained immunity is the epigenetic reprogramming that takes place upon the first stimulus, involving stable changes allowing enhanced responsiveness under subsequent stimulation. Therefore, trained immunity is defined as the entire process of

increased responsiveness as a consequence of priming event supported by epigenetic reprogramming [54, 55].

While the immunological memory of the adaptive system is based on specific gene recombination, the enhanced response to secondary stimulations characteristic of trained immunity mostly depends on epigenetic reprogramming acquired by innate cells during the first microbial encounter and it is mostly characterized in macrophages, although also described in dendritic, natural killer and innate lymphoid cells (DC, NK and ILCs) [53, 56–58]. As relevant examples, H3K4me3, H3K4me1 and H3K27ac mark active promoters and distal enhancers, while the repressive H3K9me3 and H3K27me3 are usually reduced upon the immune training process [59–62] (Figure 2c–e).

Trained immunity is a peripheral phenomenon arising at the level of mature tissue-resident myeloid cells, but it also occurs centrally, at the level of undifferentiated bone marrow (BM) haematopoietic stem cell progenitors, which epigenetic reprogramming leads to increased myeloid differentiation [63–65]. Central immune training allows the endurance of the phenotype over several months, notwithstanding the much shorter myeloid cell average half-life in circulation [66]. BM-derived macrophages from mice trained with Bacillus Calmette-Guérin (BCG) vaccination show a specific epigenetic fingerprint including H3K4me3 and H3K27ac which is able to provide more efficient protection against subsequent *Mycobacterium tuberculosis* infection [65]. A strong indication of the functional significance of long-term BM reprogramming comes from the capability of BM transplantation to transfer the trained immunity to naïve mice [64, 65]. In the periphery, training can also lead to immune suppression. In some circumstances, DCs are trained to engage higher and epigenetic-dependent transcriptional activation upon secondary stimulation; however, lung-resident DCs can acquire a tolerogenic memory after the resolution of pneumonia and cause long-term susceptibility to secondary infections [67, 68] (Figure 2c–e). Similarly, either immune activation or tolerance are induced in brain-resident macrophages via changes in H3K4me1 and H3K27ac, and HDAC1/2 function, with repercussions on cerebral inflammation and pathological grade in a mouse model of Alzheimer disease [69, 70]. Epigenetic reprogramming is key also in modulating NK cell memory and non-antigen-specific ILC priming and epigenetic changes resembling a trained phenotype have been also described in monocytes from allergic children [71–74].

An important aspect of immune training is the interlaced regulation of epigenetic reprogramming and metabolic modifications. Epigenetic regulation immediately impacts the transcription and activates the expression of metabolic enzymes, such as glycolytic hexokinase and pyruvate kinase; while metabolic activation is key for displaying full macrophagic function, it is also necessary to produce the acetyl-CoA, thus providing with the acetyl used by HATs [59]. Moreover, metabolic intermediates are directly linked to modulation of histone methylation, that is, fumarate, and some can function as methyltransferase cofactors [75–77].

There is epidemiological evidence that BCG vaccination-dependent trained immunity protects subjects of different ages from unrelated secondary infections, including SARS-CoV-2 [78–82]. Recently, Katzmarski et al. have demonstrated that trained immunity can be transmitted inter- and trans-generationally, with the progeny of trained mice (i.e., animals surviving an infective event) showing a more accessible chromatin of BM-resident granulocyte-monocyte progenitor cells on promoter regions of genes driving myeloid cell activation [83]. This work has been the first to demonstrate that infection-dependent epigenetic changes can transmit adaptive immune traits in mammals, but the observation that parental BCG vaccination associates with higher early-life survival is a very intriguing suggestion of a human 'immunological inheritance' as well [84]. Since infections dramatically impact on survival, the transgenerational inheritance of epigenetic marks linked to a better host defence may represent a strong evolutionary force in times of epidemics. In the next future, by using animal models of diseases, it will be important to assess whether the transmission of monocytic cell epigenetic fingerprint primed to mount stronger pro-inflammatory responses can also predispose the progeny to dysregulated innate immune responses and hyper-inflammatory conditions [85].

Epigenetic control of CD4$^+$ T cell fate commitment

Epigenetic mechanisms drive lymphocyte differentiation and function in response to specific developmental and environmental signals [86, 87]. Following antigenic stimulation, naïve T cells proliferate and differentiate into effector and/or memory subsets, characterized by the ability to produce specific cytokines; this is sustained and temporally guided by a coordinate network of epigenetic modifications [88]. Cytokines released in the microenvironment during T-cell receptor (TCR) activation are able to drive a signalling cascade and activate TFs, leading to CD4$^+$ Th cell polarization. Epigenetic modifications control chromatin accessibility to lineage-specific master TFs (e.g.: GATA3, T-box expressed in T cells (T-bet), retinoic acid-related orphan receptor (ROR)γt, forkhead box P (Foxp)3) driving CD4$^+$ Th cell fate commitment. Signal transducer and activator of transcription (STAT)1 signalling

pathway promotes T-bet expression in IFN-γ-activated CD4$^+$ T cells. Furthermore, T-bet increases the expression of IFN-γ and IL-12 receptors in the differentiating Th1 cells. IL-12 signalling induces STAT4 activation, which binds to the *Ifn-γ* promoter and recruits a chromatin remodelling complex namely Brahma-related gene 1 (BRG1), leading to nucleosome remodelling and increase of *Inf-γ* transcription [89] (Figure 3a). Th1 polarization is also controlled by the presence of permissive H4 acetylation mark at the *Ifn-γ* promoter, which increases in activated CD4$^+$ T cells polarized toward Th1 (under IL-12 and IL-4 stimuli) compared to Th2 or undifferentiated Th cells. In addition, T-bet also inhibits the differentiation toward other CD4$^+$ T cell subsets by repressing their master TFs, such as GATA3 and RORγt [90].

Activated Th2 cells show an increase of the H3K9ac and H3K4me3 permissive marks at *Il-4*, *Il-5* and *Il-13* gene loci [91]. The accumulation of the repressive histone mark H3K27me3 inhibits the expression of the *Ifn-γ* locus by enhancer of zeste 2 polycomb repressive complex 2 subunit (EZH2), the enzymatic subunits of polycomb-

repressive complex 2 (PRC2) that catalyzes the di- and tri-methylation of H3K27 [92] (Figure 3b). Moreover, in Th2 cells, it has been shown that an increase of the repressive histone modification H3K9me3 at Th1 *loci* leads to the silencing of Th1 cell lineage. The H3K9me3 mark is able to recruit the epigenetic reader HP1a, the major component of the transcriptional repressor complexes leading to Th1 silencing, establishing Th2 lineage stability [93]. Genome-wide analysis of H3K4me1 and p300 enhancer signature in Th1 and Th2 cells revealed a functional role for STATs proteins. It has been demonstrated that STAT6 plays a major role in p300 binding and H3K4me1 mark deposition in Th2 cells, while STAT1 and STAT4 generate active enhancer landscape in Th1 cells. These results highlight a central role of STATs proteins as environmental sensors, remodelling enhancer activity in differentiated Th cells [94].

TGF-β, IL-6, IL-21 and IL-23 are essential for naïve CD4$^+$ T cell differentiation into Th17 cells. TGF-β activates the small mother against decapentaplegic (Smad) signalling pathway, whereas IL-6 induces the activation
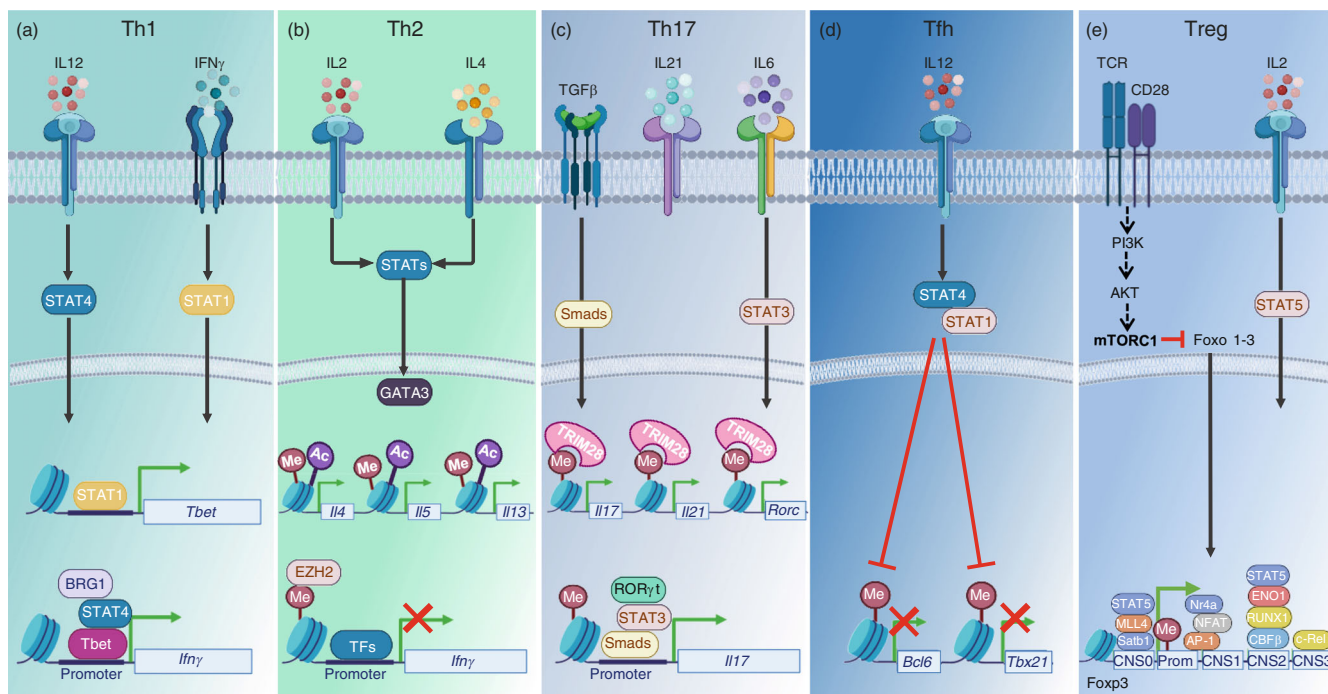

FIGURE 3 Epigenetic modifications in CD4$^+$ T helper subsets. Most relevant epigenetic modifications underlying Th1 (a), Th2 (b), Th17 (c), Tfh (D) and Treg (E) cell fate commitment. Ac, acetyl; AKT, protein kinase B; AP-1, activator protein 1; Bcl6, B-cell lymphoma 6 protein; BRG1, Brahma-related gene-1; CBFβ, mammalian core binding factor beta; CNS, conserved non-coding sequences; EZH2, enhancer of zeste homologue 2; Foxo, Forkhead box O; Foxp3, Forkhead box P3; GATA, GATA binding protein; IFN, Interferon; Me, methyl; MLL4, disordered regions of mixed lineage leukaemia 4; mTORC1, mTOR complex 1; NFAT, nuclear factor of activated T cells; Nr4a, nuclear receptor subfamily 4a; Pdcd1, programmed cell death protein 1; PI3K, phosphatidylInositol 3-kinase; ROR, retinoic-acid-receptor-related orphan nuclear receptor; RUNX, Runt-related transcription factor; Satb, special AT-rich sequence-binding protein; SMAD, small mothers against decapentaplegic; STAT, signal transducer and activator of transcription; T-bet, T-box expressed in T cells; Tbx21, T-box transcription factor; TCR, T-cell receptor; TF, transcription factor; TGF-β, transforming growth factor β; Th, T helper cell; Treg, T regulatory cell; TRIM28, tripartite motif containing 28. Figures have been created with BioRender.com.

of STAT3 that binds the *Il-17* promoter leading to an increase of the H3K4me3 permissive mark at the *Il-17* locus [95, 96] (Figure 3c). The deposition of either permissive or repressive histone marks also concerns the specific gene *loci* that regulate the expression of IL-21, another Th17-distinctive cytokine [86]. Th17 differentiation is carried out by the master TF RORγt, necessary and sufficient to induce *Il-17a* expression [97]. Jiang et al. demonstrated that the Tripartite motif containing 28 (TRIM28) expression in Th17 cells is required for the production of specific Th17 cytokines. Indeed, the binding of TRIM28 is accompanied by H3K4me3 and DNA hydroxyl-methylation (5hmc) at specific Th17 cell-related genes (*Il-17/Il-17f*, *Il-21*, *RORc*, *RORα*, *basic leucine zipper ATF-like transcription factor [Batf]* and *Irf4*) and IL-6/STAT3 signalling facilitates TRIM28 binding to the *Il-17-Il-17f* locus through the induction of permissive epigenetic events [86] (Figure 3c). Moreover, TRIM28 binds STAT3 and RORγt promoting the recruitment of RORγt to its target cytokine genes [98].

The epigenetic mechanisms underlying the specificity and plasticity of CD4+ Th cells are quite complex. Global mapping of H3K4me3 and H3K27me3 revealed a more complicated network in the lineage commitment of T-helper cell subsets. Wei et al. demonstrated that, while the signature-cytokines *loci* have a precise epigenetic mark identifying the specific CD4+ Th subset, none of the TFs 'master regulator' of the lineage commitment has a defined signature. Indeed, the TF *loci* show a 'poised' state with both H3K4me3 and H3K27me3 marks, underlying some degree of plasticity and suggesting a more dynamic regulation of naïve CD4+ Th cell differentiation [99]. The complex network of molecular mechanisms described above clearly corroborates the dominant role of epigenetics in shaping CD4+ Th cell lineage fate.

Control of gene expression by epigenetic modifications seems to play an essential role also in the development of cytokine-skewed T-follicular helper (Tfh) cells. For instance, STAT4 transduces signals from the IL-12 receptor and controls permissive H3K36me3 and H3K4me3 modifications that regulate gene *loci* important in Th1 and Tfh cell differentiation (i.e., *Bcl6*, *Pdcd1*, and *Il-21*) [100]. Moreover, Tfh1 cell differentiation, driven by IL-12, occurs through the phosphorylation of STAT1 and STAT4 proteins involved in the suppression of the histone repressive mark H3K27me3 on the *Tbx21* and *Bcl6* gene loci [101, 102] (Figure 3d). This determines IL-12-driven expression of Tfh cell-associated genes, such as *ICOS* and *Bcl-6* [102]. In addition, STAT3, which transduces signals from the IL-6 receptor, also regulates the commitment of CD4+ T cells to either a Tfh1 or Th1 phenotype by regulating T-bet expression [103]. Tfh cells

coproducing IL-17 in addition to IL-21, termed Tfh17 cells, have been described in mice and shown to share many characteristics of Th17 cells, in that their differentiation is dependent on RORγt, as well as on receptor ligation via IL-6, IL-21 and TGF-β and to expand in response to IL-23 [97, 104–108]. c-Maf, important in the induction of both *ICOS* and *Bcl-6*, leads to co-expression of IL-17 and IL-21 [104, 109, 110]. Since disrupted T-cell fate commitment is involved in a variety of pathological conditions, such as autoimmune and allergic disorders, future studies on the epigenetic mechanisms driving T-cell differentiation and function will contribute to the understanding of these diseases supporting the development of novel therapeutic strategies.

Epigenetic signature locks up immune tolerance through Foxp3

Foxp3 is essential for the generation and function of Treg cells, the CD4+ T cell subset that restrains improper and hazardous immune responses toward the *self* [111]. Foxp3+ Treg cells are either produced in the thymus (thymus-derived Treg, tTreg cells) or induced in the periphery (peripheral-derived Treg, pTreg cells) from conventional T (Tconv) cells. The establishment of the Treg cell-specific epigenetic pattern occurs before and does not depend on Foxp3 expression at early stages of tTreg cell generation [7, 112]. The *Foxp3* locus contains conserved non-coding sequences (CNSs) acting as key functional enhancers for the induction and stabilization of Foxp3 expression (Figure 3e). Genetic deletion studies have shown that CNS3 and CNS1 control the induction of *Foxp3* in the thymus and in the periphery, respectively [113]. *Foxp3* transcription is initiated after thymic CD4-single positive T cells receive strong and persistent TCR stimulation as well as CD28, IL-2 and TGF-β signals. In addition to the binding of NF-κB (c-Rel) to CNS3, the *Foxp3* promoter is bound by TCR-induced TFs, such as nuclear factor of activated T-cells (NFAT), activator protein 1 (AP-1) and nuclear receptor subfamily (Nr4a). CNS1 contains the TGF-β-response element, which activates Foxp3 transcription in a TGF-β- and TCR-dependent fashion during pTreg cell generation [113]. The recently identified *Foxp3* enhancer region (CNS0) contributes to the induction of *Foxp3* both in the thymus and in the periphery, through specific changes in chromatin conformation and gene looping. Located upstream of the transcription start site, it binds the HMT KMT2D (also known as MLL4) which increases the H3K27me1, distinctive of primed and active enhancers. Moreover, other TFs, such as the genome organizer Satb1, the non-canonical BAF chromatin-modifying complex component

BRD9 and STAT5 also bind CNS0; through chromatin looping, TCR-induced TFs may interact with the distal enhancers to form a *Foxp3*-inducing enhanceosome [114]. In addition, CNS0- and CNS3-double deletion almost completely abrogates tTreg and pTreg cell development due to decreased stability of *Foxp3* gene [115].

The demethylation of CpG islands in Treg cell-specific demethylation region (TSDR) of mouse and human effector Treg cells associates with stable Foxp3 expression. This region is contained in the CNS2 and, when demethylated, is bound by Foxp3, RUNX1-core binding factor (CBF)-β and STAT5. Intriguingly, CNS2 demethylation begins after Foxp3 transcription has started and Foxp3 itself may concur to this DNA demethylation process. Indeed, after several cell divisions, CNS2-deficient Treg cells lose Foxp3, indicating CNS2 importance for its maintenance [115]. This process underlines two regulatory checkpoints in Treg cell differentiation and stability: the initial transcriptional activation of Foxp3 and the subsequent CNS2 demethylation, required to establish faithful epigenetic memory of Foxp3 expression and secure Treg cell lineage commitment. In addition, through the binding of STAT5 to CNS2, IL-2 signalling may strengthen Foxp3 transcription [113] (Figure 3e). However, Foxp3 expression can be tuned in response to different environmental cues, particularly in conditions of limiting extracellular IL-2 or glucose availability. Indeed, IL-2 shows two mechanisms of enhancing Foxp3 expression: via the activation of the CNS2 enhancer and via the repression of the *Foxp3* long intergenic noncoding RNA (Flicr), which modifies chromatin accessibility in the CNS3 region and negatively tunes Foxp3 expression [116]. Furthermore, the binding of the glycolytic enzyme enolase-1/myc-binding protein-1 (ENO-1/MBP-1) to *Foxp3* promoter and CNS2 during glycolysis inhibition has been shown to determine its transcriptional repression during iTreg cell generation [117] (Figure 3e).

Overall, despite the well-known epigenetic regulation at the *Foxp3* locus, the underlying mechanisms that regulate the stability of the Treg cell pool remain unclear, particularly the role of DNA methylation in sealing their fate. Recently, the epigenetic regulator ubiquitin-like with plant homeodomain and RING finger domains 1 (Uhrf1), which regulates de novo DNA methylation via the recruitment of DNMT3a and DNMT3b, has been shown to control the maintenance of DNA methylation at inflammatory gene *loci*, essential for stabilizing the identity and suppressive function of mature Treg cells. Indeed, despite preserving Foxp3 expression and methylation pattern, Foxp3$^+$ Treg cells from *Uhrf1* chimeric knockout mice exhibit down-regulation of genes associated with Treg-suppressive function [118]. In all, the summarized evidence from the literature underlines how sophisticated is the epigenetic control of *Foxp3* in the complex scenario that governs T cell fate, from the thymus to the acquired regulation in the periphery.

T regulatory cell plasticity as novel regulator of immune tolerance

There is increasing evidence that the loss of Foxp3 is the molecular driver of Treg cell plasticity, defined as the attitude to convert into potentially pro-inflammatory Th cell subsets. At the same time, Foxp3 expression is sufficient to completely reprogram T cells from a pro-inflammatory to a suppressive phenotype. Indeed, thanks to its cooperative interaction with TFs that affect gene expression through chromatin modification, Foxp3 alters the expression of genes encoding for pro-inflammatory cytokines and Th-lineage TFs. Indeed, it interacts with NFAT, Eosinophilia familial (Eos), acute myeloid leukaemia 1 protein (AML1), GATA-3, RORγt as well as with chromatin modifiers such as the class II HDAC7-9 and the HAT tat-interactive protein (TIP)-60 [119]. In humans, *Foxp3* gene encodes for different splicing variants. The full-length (Foxp3fl) transcript and those lacking the region encoded by *exon 2* (Foxp3Δ2) are the most abundant isoforms, while forms lacking the region encoded by *exon 7* (Foxp3Δ7 and Foxp3Δ2Δ7) are less frequent [120]. Despite their role has not been completely addressed, it is evident that Foxp3 interaction with specific cofactors is affected when the splicing event impairs the relative binding domain. Indeed, the *Foxp3 exon2* (Foxp3E2) encodes the protein domain responsible for the binding to RORα and RORγt; as a consequence, Foxp3Δ2 does not confer proper suppressive ability to Treg cells [121]. Similarly, loss of the *exon7* (E7), encoding part of the leucine-zipper domain, alters homo- and hetero-association of Foxp3 and its DNA binding [122]. In addition, the different Foxp3 domains fulfil discernible functions in gene regulation. More in detail, most Foxp3-regulated genes are affected by loss of the proline-rich (ProR) domain involved in the recruitment of class I HDACs to Foxp3 target genes, such as *Il-2* and *Ifnγ*, where deacetylation establishes silent chromatin during Treg cell fate commitment. The regulatory mechanism of the ProR domain involves the subdomain encoded by exon 1 and the four amino-acid motifs within exon 2 (m4.2) [119]. Treg cells that have lost Foxp3 expression—hence defined 'ex-Foxp3'—mostly convert into Th2-like cells upon in vitro stimulation under nonpolarizing conditions, as they overexpress several Th2-specific genes, such as *Il-4*, *Il-5* and *Il-13*, the transcription factor *GATA3* and the surface receptor *G*

protein-coupled receptor (GPR)44. Multiple studies have suggested that, during infection, exposure of Treg cells to pro-inflammatory cytokines (such as TGF-β and IL-6) drives Foxp3 loss and conversion into Th17-like effector cells, to facilitate appropriate immune responsiveness. On the contrary, the molecular mechanism governing Treg cell resilience to inflammation-induced *Foxp3* destabilization remains elusive. Intriguingly, Li et al. identified methyl-CpG binding protein 2 (MeCP2) as a crucial player in the epigenetic machinery that confers Treg cell stability during inflammation. Indeed, MeCP2 is specifically recruited to the CNS2 region, where it collaborates with cAMP responsive element binding protein 1 (Cpb1) to promote H3ac, thereby counteracting inflammation-induced epigenetic silencing of *Foxp3* [123]. However, an important aspect that needs to be considered is the presence of non-suppressive Foxp3$^+$ T cells in the immune system and loss of Foxp3 in Treg cells under certain conditions; therefore, functional Treg cells can be more accurately defined as the T cell subset holding the Treg-cell type epigenome, rather than Foxp3 alone. This epigenome-based definition of Treg cells would enable better understanding of functional stability, plasticity, and heterogeneity of Treg cells, in both physiological and pathological conditions.

Epigenetic mechanisms underlying CD8$^+$ T cell functions

Transcriptional and epigenetic regulation has been described as a major actor in early CD8$^+$ effector and memory cell fate decisions. While *DNMT3a* knockout CD8$^+$ T cells maintain their effector function, there is a strong increase in the development of memory precursors; this is secondary to the ineffective repression of the transcription factor T-cell factor 1 (Tcf1) due to the lack of DNMT3a binding to the *Tcf1* promoter (Figure 4a). Therefore, DNMT3a is considered a decisive regulator that fine-tunes early effector/or memory fate decisions [124]. It is worth noticing that CD8$^+$ cytotoxic T cells terminate CD4 transcription by up-regulating *RUNX3*, which binds the identical *cis*-element as RUNX1. Verbaro et al. demonstrated that the HMT G9a is required for CD8$^+$ T cell development in non-inflammatory conditions. Indeed, it interacts with RUNX3 leading to the silencing of genes involved in Th cell commitment; the deletion of *G9a* in T cells is able to reactivate the expression of several genes implicated in CD4$^+$ T cell lineage decision [125] (Figure 4a). Moreover, Tsao et al. evaluated the transcriptome and epigenome of differentiating CD8$^+$ T cells in mice, highlighting the central role of BATF. Through genome-scale profiling, they observed
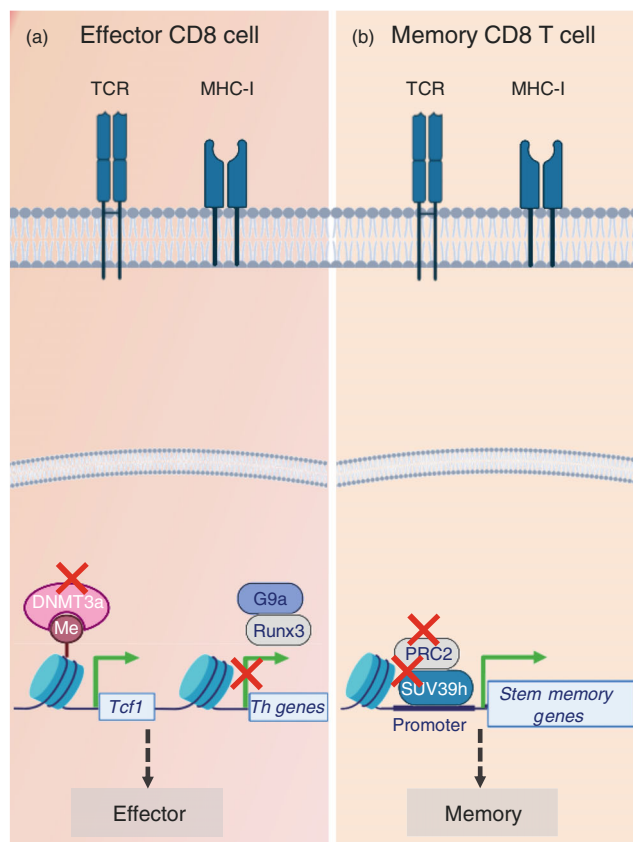

FIGURE 4 Epigenetic modifications in effector/memory CD8$^+$ cells. Epigenetic events that regulate effector (a) and memory (b) CD8$^+$ cells. DNMT, DNA methyltransferase; G9a, G9a methyltransferase; Me, methyl; MHC, major histocompatibility complex; PRC2, polycomb repressive complex 2; RUNX, Runt-related transcription factor; Tcf1, T-cell factor 1; TCR, T-cell receptor. Figures have been created with BioRender.com.

that BATF induces transcriptional changes in stimulated naïve cells and establishes the effector cell transcriptional and epigenetic program through the cooperation with a network of TFs (Irf4, RUNX3 and T-bet) [126]. The HMT SUV39h is responsible for H3K9me3, driving transcriptional silencing [127]. Together with SUV39h, PRC2 is induced during CD8$^+$ T cell activation, leading to repression and regulation of effector/memory differentiation. Since SUV39h1 plays a critical role in the establishment of the chromatin marks that silence stem/memory genes during CD8$^+$ T effector differentiation, *SUV39h1*-defective cells show increased long-term memory reprogramming capacity [128] (Figure 4b). Recently, the protein arginine methyltransferase-1 (PRMT1) has been shown to epigenetically control and enhance CD8$^+$ T cell polyfunctionality, as the ability to produce IL-2. PRMT1 determines an increase of the permissive transcription marker H4K3me2 at the *Il-2* promoter following Wingless-related integration site (Wnt) activation [129].

In all, these data underline the contribution of the epigenetic changes in the regulation of CD8$^+$ effector/memory differentiation.

Epigenetic control of B cell activation

Naïve B cells display an inactive epigenetic profile characterized by a genome-wide DNA hypermethylation and histone deacetylation [130], except for B-cell lineage genes, such as *CD19*, *Pax5*, *Ebf1* and *Spib*, appearing in an active epigenetic state [131]. B-cell activation, occurring through the B-cell receptor (BCR) and the binding of the Toll-like receptor (TLR) to antigenic epitopes and pathogen-associated molecular patterns (PAMPs), leads to the induction of H3K4me3, H3K9ac and H3K14ac to the promoter regions of *activation-induced cytidine deaminase (AID)*, involved in DNA methylation dynamics of the germinal centre (GC) B cells [132, 133](Figure 5a). Epigenetic modifications have also been considered crucial during immunoglobulin (Ig) class switch DNA recombination and somatic hypermutation, through a CREB binding protein (CREBBP)- and AID-dependent mechanism [134, 135]. Plasma cells display a transcriptional signature distinct from that of B cells, due to the acquisition of permissive histone modifications, including

H3K4me1 and H3K4me4 in active promoters and distal enhancers [136, 137]. Overexpression of Blimp-1 (encoded by *Prdm1*) in peripheral mature B cells promotes antibody production, whereas *Blimp-1*-specific deletion in plasma cells leads to the loss of antibody production despite the retention of the plasma cell-related transcriptional markers [138]. Blimp-1 is a transcriptional repressor with a DNA-binding activity conferred by five zinc-finger motifs and requires the association with histone deacetylases and hGroucho to induce transcriptional repression [139–141]. The differentiation of activated B cells into plasma cells requires coordinated changes in the expression of many genes, including the silencing of B cell-associated transcripts encoding the TFs Pax5, Bach2 and Bcl-6, and the activation of a plethora of plasma cell-specific genes [136, 142]. In activated B cells, Bcl-6, metastasis-associated 1 family member (MTA)3, Pax5 and microphthalmia-associated transcription factor (MITF) are important to repress plasma cell formation. On the contrary, in plasma cells, *Bcl-6*, *MTA3*, *Pax5* and *MITF* are repressed by the coordinated action of Blimp1, X-box-binding protein (XBP)1 and IRF4, leading to the silencing of the B-cell gene-expression program [142, 143]. In particular, Blimp-1 induces histone deacetylation in the promoter regions of *Bcl6*, *Pax5* and *Spib* genes allowing a low histone acetylation levels in plasma cells

FIGURE 5 Epigenetics in B-cell activation. B-cell function is regulated by distinct epigenetic events leading to determine naïve (a), plasma cell (b) and memory (c) states. Ac, acetyl; Aid, activation-induced deaminase; Bcl6, B-cell lymphoma 6 protein; BCR, B-cell receptor; Blimp1, B-lymphocyte-induced maturation protein 1; C-myc, C-myelocytomatosis oncogene; EZH2, enhancer of zeste homologue 2; G9a, G9a methyltransferase; IRF4, Interferon regulatory factor 4; Me, methyl; Pax5, paired box 5; TFs, transcription factors; TLR, toll-like receptor; Ub, Ubiquitin; XBP1, X-box binding protein 1. Figures have been created with BioRender.com.
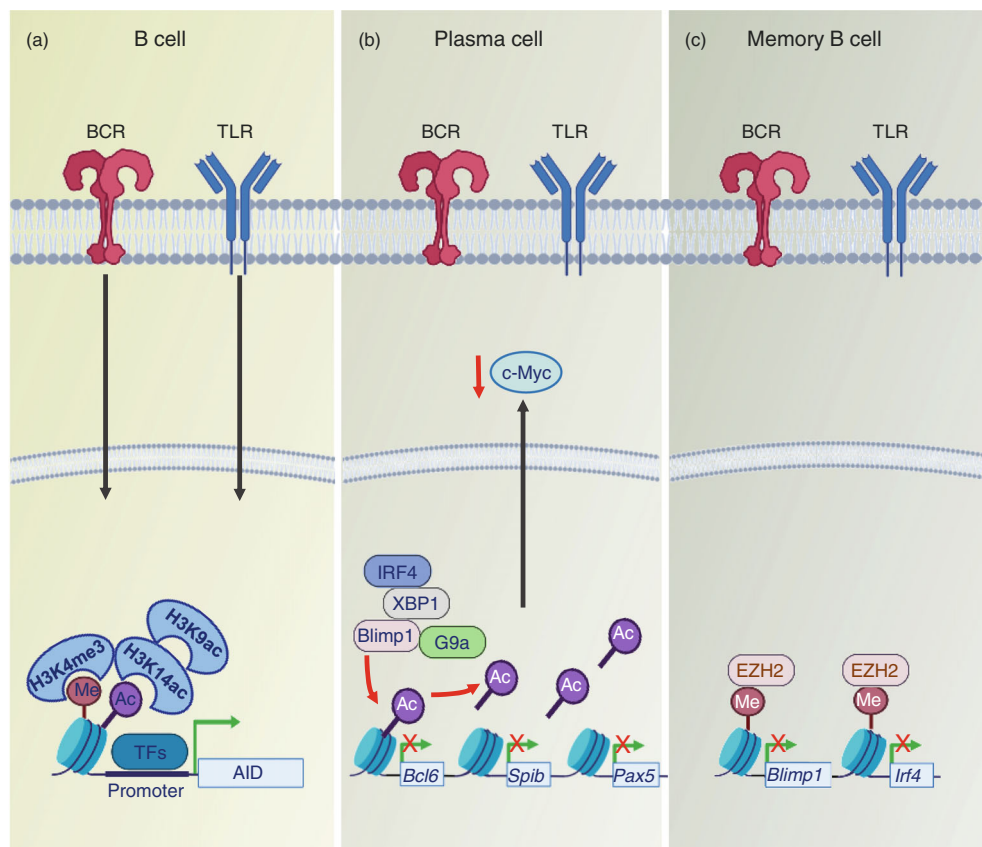
together with a decreased c-Myc expression [139] (Figure 5b). Furthermore, Blimp-1 has been found to bind to H3K9MT G9a, driving its recruitment to the promoter regions of *Spib* and *Pax5*, leading to gene silencing [144]. Histone modifications affect the hallmark genes of memory B cells, such as *CD38* in mouse and *CD27* in human [135, 145]. Quiescent memory B cells display reduced lysine methylation levels compared with active memory B cells [146]. EZH2, which catalyses H3K27me3, is highly expressed in human GC B cells, where it represses *Blimp1* and *Irf4* expression to constrain terminal B-cell differentiation induced by IL-21 (Figure 5c). Through the chromatin silencing at these gene loci, EZH2 ensures the persistence of B cells in the GC reaction, enabling the generation of high-affinity antibodies and memory B cells. The inhibition of EZH2 in murine GC B cells causes a profound impairment of memory B cell formation and dramatically affects humoral immunity [147].

The epigenetic code of autoimmune disorders

Loss of immune tolerance in autoimmune disorders does not seem to be triggered by specific genetic mechanisms, although they can confer susceptibility to those diseases [148]. As described above, the dynamic control of the chromatin conformation plays a key role in the regulation of lymphocyte commitment and functionality, and therefore impacts on the induction of the autoimmune attack [149]. Recent studies have demonstrated that epigenetic changes may be crucial in clinical manifestations of autoimmune diseases such as SLE, systemic sclerosis (SSc), multiple sclerosis (MS), rheumatoid arthritis (RA), Sjogren's syndrome (SS), autoimmune thyroid disease (AITD) and type 1 diabetes (T1D); this is also confirmed by the discordant onset rate in monozygotic twins [150–154]. Several epigenetic drugs (Epidrugs), like HDAC or DNMT inhibitors (HDACi or DNMTi, respectively), are now under investigations as promising therapeutic tools in autoimmunity, also in combination with other pharmacological agents [155, 156] (Table 1).

In SLE, the hypomethylation of genes, such as *CD11a*, *perforin*, *CD70* and *CD40L*, seems to contribute to their overexpression that drives CD4+ T cell autoreactivity [157, 158] and treatment with DNMTi has been shown to induce a lupus-like syndrome in a murine model [159–161]. Again, H3 modifications appear to be prevalent in SLE patients as testified by the augmented levels of H3K27me3 and H3 hypoacetylation in CD4+ T cells that correlate with active disease [157, 162], reversible by administration of HDACi such as trichostatin A

(TSA) [163] (Table 1). An unbalance of histone acetylase/deacetylase activity has been reported by Huber et al. also in synovial tissues of patients with RA [164]. Histone modifications are also involved in the process of angiogenesis, of paramount importance in the maintenance of synovial tissue inflammation, as testified by the therapeutic effect of an HDACi—FK228—occurring through the down-regulation of specific angiogenic-related factors, namely Hypoxia-induced factor-1α (HIF-1 α) and Vascular Endothelial Growth Factor (VEGF) [165]. Lastly, another HDACi—Largazole—has been shown to reduce the expression of intracellular adhesion molecule-1 (ICAM-1) and the vascular adhesion molecule-1 (VCAM-1) which control leukocyte migration to the inflammatory joints [166] (Table 1). The effect of HDAC1i has been confirmed in juvenile idiopathic arthritis, in which HDAC1i—Givinostat—improves the clinical picture with an excellent safety profile and, thus, supports the contribution of histone modification to the onset of inflammation in this autoimmune disorder [167].

In patients with SSc, DNMT downregulation has been reported to support the overexpression of several genes involved in disease progression, such as *CD40L*, *CD11a* and *CD70* [168–170]. Moreover, hypomethylation of the type I IFN signalling pathway-associated genes, such as *myxoma resistance protein (MX)1*, *interferon-induced protein 44 like (IFI44L)*, *Poly (ADP-Ribose) polymerase (PARP)*, *STAT1* and *ubiquitin-specific peptidase (USP)8*, has been reported in both CD4+ and CD8+ T cells of SSc patients [171]. Furthermore, reduced acetylation levels in both H3 and H4 of SSc fibroblasts have been demonstrated [172, 173]. Treatment with TSA seems to reduce fibrosis and collagen expression in SSc fibroblasts cultured in vitro [174]. Recently, the evolutionarily conserved Wnt pathway, which regulates crucial aspects of cell fate determination during embryogenesis, has also been reported to increase skin fibrosis in SSc patients via epigenetic dysregulation; treatment with 5-azacitidine (5-Aza) abolishes the fibrotic phenotype [175–177]. Noteworthy, in peripheral blood cells of SS patients, the same hypomethylation of the type I IFN signalling pathway-associated genes is coupled with hypermethylation of *RUNX1* and *Foxp3* genes, important in the control of Treg cell generation and function [178, 179] (Table 1).

Recent studies have highlighted the correlation between the hypomethylation of specific genes and the pathogenesis of MS. For example, the *peptidyl arginine deiminase (PAD)* promoter types II and IV, involved in the process of citrullination of the basic myelin protein (MBP); the *Il-17a* promoter that correlates with increased numbers of Th17 cells; the *HLA-DRB1* locus and several genes involved in oxidative stress, hippocampal atrophy and neuronal differentiation [180–182]. Moreover,

TABLE 1 Autoimmune disorders and the underlying epigenetic alterations, with reference to their target genes or cell types and the epigenetic drugs (epidrugs) mitigating their phenotypes, as reviewed in the text

Autoimmune disorder	Epigenetic alteration	Target/cell type	Epidrug	References
SLE	DNA methylation	*ITGAL*; *PRF1*; *TNFSF5*; *TNFSF7*	5-azacytidine, procainamide, hydralazine	[158, 191, 192]
	Histone modification	T cells	Trichostatin A	[163, 193, 194]
RA	Histone modification	Synovial fibroblasts and T cells	Givinostat	[167, 195]
		HIF1α and *VEGF* in synovial fibroblasts	FK228	[165]
		TNF-α pathway in synovial fibroblasts	Largazole	[166]
SSc	DNA methylation	*CD40L*; *CD11a*; *CD70* Wnt pathway *MX1*; *IFI44L*; *PARP*; *STAT1*; *IFI44L*; *USP8*	5-azacytidine	[171, 176, 177]
	Histone modification	Fibroblasts	Trichostatin A	[174]
SS	DNA methylation	*MX1*; *IFI44L*; *PARP*; *STAT1*; *FI44L*; *USP8*; *RUNX1*; *Foxp3*; *PAD2*; *PAD4*; *Il-17A*	5-azacytidine	[178, 196]
MS	DNA methylation	*HLA-DRB1*; *ARSB*; *KCTD11*; *Foxp3*		[180–183]
AITD	DNA methylation	T cells		[184]
GD	Histone modification	T cells		[185]
T1D	DNA methylation	*Foxp3*; *Il-4*; *Il-18*; *Il-22*; *Il-23*; *Il-27p28*	Trichostatin A	[188]

Abbreviations: AITD, autoimmune thyroid disease; GD, Graves' disease; MS, multiple sclerosis; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SS, Sjogren's syndrome; SSc, systemic sclerosis; T1D, type 1 diabetes.

hypermethylation of the *Foxp3* promoter has been described in experimental autoimmune encephalomyelitis (EAE) mice [183] (Table 1).

Finally, a global DNA hypomethylation has been reported in AITD, due to genetic polymorphisms of regulatory genes, such as *DNMT1* or *methionine synthase reductase* (*MTRR*) [184]. In addition, Graves' disease (GD) patients show elevated levels of H3K4me3 and H3K27Ac in CD4$^+$ and CD8$^+$ T cells [185]. In T1D patients, *Foxp3* hypermethylation in CD4$^+$ T cells results in its reduced expression and altered Treg cell generation [186]. Furthermore, high levels of H3K9me2 in the *cytotoxic T-lymphocyte antigen 4* (*CTLA4*) promoter have been reported during T-cell activation [187]. Finally, recent evidence exists that the use of TSA in mice may protect from T1D through the epigenetic modulation of *Foxp3*, *Il-22* and *Il-23* in the pancreas and of genes encoding for *Il-4*, *Il-18*, *Il-23* and *Il-27p28* in splenic lymphocytes [188] (Table 1).

In addition to the epigenetic modifications of specific target genes, their regulation also involves dynamic communication between promoters and several distant enhancers allowing a reliable transfer of regulatory information over distance. As reported in a recent study based on a new approach for fine mapping causal genetic variants for 21 autoimmune disorders, most of causal determinants are represented by a very specific subset of enhancers involved in T-cell stimulation [189]. Mumbach

et al. used the histone modification correlating with active enhancers and promoters (H3K27ac) as a bait in their recently developed HiChIP method, to map protein-centric chromatin interactions. In this way, they were able to obtain high-resolution maps of enhancer–promoter contacts in primary naïve CD4$^+$, Treg and Th17 cells; they identified several chromatin loops shared by all three cell types with the 91% of the loop anchors associated with either an enhancer or a promoter. They found that most of disease-associated enhancers are able to contact other genes beyond the nearest in the genome, increasing the number of potential target genes for autoimmune and cardiovascular diseases [190].

Taken together, these findings underline how defects in the epigenetic control of immune cell function can concur to autoimmune disease pathogenesis and progression.

CONCLUDING REMARKS

Healthy immunity relies on the immune cell ability to finely tune in a constantly changing environment. This ability is guaranteed by the functional diversity of the differentiated immune cells and by their high level of plasticity, considered as the capacity to adapt to the extracellular milieu. Nonetheless, either failure to adapt

or loss of cell homeostasis can trigger an exaggerated immune response supporting the development of immune-related disorders. This research field has significantly advanced through the knowledge that epigenetic mechanisms support cell diversification while maintaining immune system integrity. Moreover, what is now emerging is that epigenetic mechanisms, already thought to dictate the memory of the environmental stimuli, may also contribute to the persistence of disease-associated phenotypes. It seems reasonable to attenuate pro-inflammatory responses by pharmacological 'removal' of the diseased-epigenetic modification, followed by restoration of the healthy gene expression pattern. In this context, several epidrugs are now under investigation to restore immune tolerance, as promising tools for future clinical trials in human autoimmune disorders. The complete understanding of the epigenetic underpinnings during immune cell differentiation and acquisition of cell stability will shed more light into their pathological dysregulation and help to delineate novel therapeutic strategies to halt immune disorders.

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DATA AVAILABILITY STATEMENT

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REFERENCES

1. Tammen SA, Friso S, Choi SW. Epigenetics: the link between nature and nurture. Mol Aspects Med. 2013;34(4):753–64.
2. Deans C, Maggert KA. What do you mean, "epigenetic"? Genetics. 2015;199(4):887–96.
3. Meier K, Recillas-Targa F. New insights on the role of DNA methylation from a global view. Front Biosci (Landmark Ed). 2017;22(4):644–68.
4. Li Y, Schulz VP, Deng C, Li G, Shen Y, Tusi BK, et al. Setd1a and NURF mediate chromatin dynamics and gene regulation during erythroid lineage commitment and differentiation. Nucleic Acids Res. 2016;44(15):7173–88.
5. Boller S, Ramamoorthy S, Akbas D, Nechanitzky R, Burger L, Murr R, et al. Pioneering activity of the C-terminal domain of EBF1 shapes the chromatin landscape for B cell programming. Immunity. 2016;44(3):527–41.
6. Di Stefano B, Sardina JL, van Oevelen C, Collombet S, Kallin EM, Vicent GP, et al. C/EBPalpha poises B cells for rapid reprogramming into induced pluripotent stem cells. Nature. 2014;506(7487):235–9.
7. Ohkura N, Sakaguchi S. Transcriptional and epigenetic basis of Treg cell development and function: its genetic anomalies or variations in autoimmune diseases. Cell Res. 2020;30(6):465–74.
8. Gibney ER, Nolan CM. Epigenetics and gene expression. Heredity (Edinb). 2010;105(1):4–13.
9. Fraga MF, Ballestar E, Paz MF, Ropero S, Setien F, Ballestar ML, et al. Epigenetic differences arise during the lifetime of monozygotic twins. Proc Natl Acad Sci U S A. 2005;102(30):10604–9.
10. Raghuraman S, Donkin I, Versteyhe S, Barres R, Simar D. The emerging role of epigenetics in inflammation and Immunometabolism. Trends Endocrinol Metab. 2016;27(11):782–95.

11. Li Y. Epigenetic mechanisms link maternal diets and gut microbiome to obesity in the offspring. Front Genet. 2018; 9:342.

12. Felix JF, Cecil CAM. Population DNA methylation studies in the Developmental Origins of Health and Disease (DOHaD) framework. J Dev Orig Health Dis. 2019;10(3):306–13.

13. Heijmans BT, Tobi EW, Stein AD, Putter H, Blauw GJ, Susser ES, et al. Persistent epigenetic differences associated with prenatal exposure to famine in humans. Proc Natl Acad Sci U S A. 2008;105(44):17046–9.

14. Tobi EW, Lumey LH, Talens RP, Kremer D, Putter H, Stein AD, et al. DNA methylation differences after exposure to prenatal famine are common and timing- and sex-specific. Hum Mol Genet. 2009;18(21):4046–53.

15. Tobi EW, Slieker RC, Stein AD, Suchiman HE, Slagboom PE, van Zwet EW, et al. Early gestation as the critical time-window for changes in the prenatal environment to affect the adult human blood methylome. Int J Epidemiol. 2015;44(4): 1211–23.

16. Jousse C, Parry L, Lambert-Langlais S, Maurin AC, Averous J, Bruhat A, et al. Perinatal undernutrition affects the methylation and expression of the leptin gene in adults: implication for the understanding of metabolic syndrome. FASEB J. 2011; 25(9):3271–8.

17. Jia Y, Cong R, Li R, Yang X, Sun Q, Parvizi N, et al. Maternal low-protein diet induces gender-dependent changes in epigenetic regulation of the glucose-6-phosphatase gene in newborn piglet liver. J Nutr. 2012;142(9):1659–65.

18. Wankhade UD, Zhong Y, Kang P, Alfaro M, Chintapalli SV, Thakali KM, et al. Enhanced offspring predisposition to steatohepatitis with maternal high-fat diet is associated with epigenetic and microbiome alterations. PLoS One. 2017;12(4):e0175675.

19. Varricchi G, Bencivenga L, Poto R, Pecoraro A, Shamji MH, Rengo G. The emerging role of T follicular helper (TFH) cells in aging: influence on the immune frailty. Ageing Res Rev. 2020;61:101071.

20. Jasiulionis MG. Abnormal epigenetic regulation of immune system during aging. Front Immunol. 2018;9:197.

21. Dozmorov MG, Coit P, Maksimowicz-McKinnon K, Sawalha AH. Age-associated DNA methylation changes in naive CD4(+) T cells suggest an evolving autoimmune epigenotype in aging T cells. Epigenomics. 2017;9(4):429–45.

22. Hnisz D, Abraham BJ, Lee TI, Lau A, Saint-Andre V, Sigova AA, et al. Super-enhancers in the control of cell identity and disease. Cell. 2013;155(4):934–47.

23. Sen DR, Kaminski J, Barnitz RA, Kurachi M, Gerdemann U, Yates KB, et al. The epigenetic landscape of T cell exhaustion. Science. 2016;354(6316):1165–9.

24. Fraschilla I, Amatullah H, Jeffrey KL. One genome, many cell states: epigenetic control of innate immunity. Curr Opin Immunol. 2022;75:102173.

25. Locati M, Mantovani A, Sica A. Macrophage activation and polarization as an adaptive component of innate immunity. Adv Immunol. 2013;120:163–84.

26. Hoeksema MA, de Winther MP. Epigenetic regulation of monocyte and macrophage function. Antioxid Redox Signal. 2016;25(14):758–74.

27. Jain N, Shahal T, Gabrieli T, Gilat N, Torchinsky D, Michaeli Y, et al. Global modulation in DNA epigenetics during pro-inflammatory macrophage activation. Epigenetics. 2019;14(12):1183–93.

28. Rodriguez-Ubreva J, Garcia-Gomez A, Ballestar E. Epigenetic mechanisms of myeloid differentiation in the tumor microenvironment. Curr Opin Pharmacol. 2017;35:20–9.

29. Larionova I, Kazakova E, Patysheva M, Kzhyshkowska J. Transcriptional, epigenetic and metabolic programming of tumor-associated macrophages. Cancers (Basel). 2020;12(6): 1411.

30. Xu G, Liu G, Xiong S, Liu H, Chen X, Zheng B. The histone methyltransferase Smyd2 is a negative regulator of macrophage activation by suppressing interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-alpha) production. J Biol Chem. 2015;290(9):5414–23.

31. Cheng C, Huang C, Ma TT, Bian EB, He Y, Zhang L, et al. SOCS1 hypermethylation mediated by DNMT1 is associated with lipopolysaccharide-induced inflammatory cytokines in macrophages. Toxicol Lett. 2014;225(3):488–97.

32. Chen S, Yang J, Wei Y, Wei X. Epigenetic regulation of macrophages: from homeostasis maintenance to host defense. Cell Mol Immunol. 2020;17(1):36–49.

33. Wang X, Cao Q, Yu L, Shi H, Xue B, Shi H. Epigenetic regulation of macrophage polarization and inflammation by DNA methylation in obesity. JCI Insight. 2016;1(19):e87748.

34. Li T, Garcia-Gomez A, Morante-Palacios O, Ciudad L, Ozkaramehmet S, Van Dijck E, et al. SIRT1/2 orchestrate acquisition of DNA methylation and loss of histone H3 activating marks to prevent premature activation of inflammatory genes in macrophages. Nucleic Acids Res. 2020;48(2):665–81.

35. Tran K, Risingsong R, Royce DB, Williams CR, Sporn MB, Pioli PA, et al. The combination of the histone deacetylase inhibitor vorinostat and synthetic triterpenoids reduces tumorigenesis in mouse models of cancer. Carcinogenesis. 2013; 34(1):199–210.

36. Guerriero JL, Sotayo A, Ponichtera HE, Castrillon JA, Pourzia AL, Schad S, et al. Class IIa HDAC inhibition reduces breast tumours and metastases through anti-tumour macrophages. Nature. 2017;543(7645):428–32.

37. Sun R, Hedl M, Abraham C. Twist1 and Twist2 induce human macrophage memory upon chronic innate receptor treatment by HDAC-mediated deacetylation of cytokine promoters. J Immunol. 2019;202(11):3297–308.

38. Amit I, Winter DR, Jung S. The role of the local environment and epigenetics in shaping macrophage identity and their effect on tissue homeostasis. Nat Immunol. 2016;17(1):18–25.

39. Lavin Y, Winter D, Blecher-Gonen R, David E, Keren-Shaul H, Merad M, et al. Tissue-resident macrophage enhancer landscapes are shaped by the local microenvironment. Cell. 2014;159(6):1312–26.

40. Ghisletti S, Barozzi I, Mietton F, Polletti S, De Santa F, Venturini E, et al. Identification and characterization of enhancers controlling the inflammatory gene expression program in macrophages. Immunity. 2010;32(3):317–28.

41. Gosselin D, Link VM, Romanoski CE, Fonseca GJ, Eichenfield DZ, Spann NJ, et al. Environment drives selection and function of enhancers controlling tissue-specific macrophage identities. Cell. 2014;159(6):1327–40.

42. Varricchi G, de Paulis A, Marone G, Galli SJ. Future needs in mast cell biology. Int J Mol Sci. 2019;20(18):4397.

43. Varricchi G, Galdiero MR, Marone G, Granata F, Borriello F, Marone G. Controversial role of mast cells in skin cancers. Exp Dermatol. 2017;26(1):11–7.

44. Varricchi G, Rossi FW, Galdiero MR, Granata F, Criscuolo G, Spadaro G, et al. Physiological roles of mast cells: collegium internationale allergologicum update 2019. Int Arch Allergy Immunol. 2019;179(4):247–61.

45. Melo FR, Wallerman O, Paivandy A, Calounova G, Gustafson AM, Sabari BR, et al. Tryptase-catalyzed core histone truncation: a novel epigenetic regulatory mechanism in mast cells. J Allergy Clin Immunol. 2017;140(2):474–85.

46. Balasubramani A, Larjo A, Bassein JA, Chang X, Hastie RB, Togher SM, et al. Cancer-associated ASXL1 mutations may act as gain-of-function mutations of the ASXL1-BAP1 complex. Nat Commun. 2015;6:7307.

47. Montagner S, Leoni C, Emming S, Della Chiara G, Balestrieri C, Barozzi I, et al. TET2 regulates mast cell differentiation and proliferation through catalytic and non-catalytic activities. Cell Rep. 2016;15(7):1566–79.

48. De Vita S, Schneider RK, Garcia M, Wood J, Gavillet M, Ebert BL, et al. Loss of function of TET2 cooperates with constitutively active KIT in murine and human models of mastocytosis. PLoS One. 2014;9(5):e96209.

49. Marcella S, Petraroli A, Braile M, Parente R, Ferrara AL, Galdiero MR, et al. Vascular endothelial growth factors and angiopoietins as new players in mastocytosis. Clin Exp Med. 2021;21(3):415–27.

50. Traina F, Visconte V, Jankowska AM, Makishima H, O'Keefe CL, Elson P, et al. Single nucleotide polymorphism array lesions, TET2, DNMT3A, ASXL1 and CBL mutations are present in systemic mastocytosis. PLoS One. 2012;7(8):e43090.

51. Leoni C, Montagner S, Rinaldi A, Bertoni F, Polletti S, Balestrieri C, et al. Dnmt3a restrains mast cell inflammatory responses. Proc Natl Acad Sci U S A. 2017;114(8):E1490–E9.

52. Yang IV, Lozupone CA, Schwartz DA. The environment, epigenome, and asthma. J Allergy Clin Immunol. 2017;140(1):14–23.

53. Netea MG, Quintin J, van der Meer JW. Trained immunity: a memory for innate host defense. Cell Host Microbe. 2011;9(5):355–61.

54. Fanucchi S, Dominguez-Andres J, Joosten LAB, Netea MG, Mhlanga MM. The intersection of epigenetics and metabolism in trained immunity. Immunity. 2021;54(1):32–43.

55. D'Urso A, Brickner JH. Mechanisms of epigenetic memory. Trends Genet. 2014;30(6):230–6.

56. O'Sullivan TE, Sun JC, Lanier LL. Natural killer cell memory. Immunity. 2015;43(4):634–45.

57. Placek K, Schultze JL, Netea MG. Immune memory characteristics of innate lymphoid cells. Curr Opin Infect Dis. 2019;32(3):196–203.

58. Netea MG, Dominguez-Andres J, Barreiro LB, Chavakis T, Divangahi M, Fuchs E, et al. Defining trained immunity and its role in health and disease. Nat Rev Immunol. 2020;20(6):375–88.

59. Saeed S, Quintin J, Kerstens HH, Rao NA, Aghajanirefah A, Matarese F, et al. Epigenetic programming of monocyte-to-macrophage differentiation and trained innate immunity. Science. 2014;345(6204):1251086.

60. Arts RJW, Carvalho A, La Rocca C, Palma C, Rodrigues F, Silvestre R, et al. Immunometabolic pathways in BCG-induced trained immunity. Cell Rep. 2016;17(10):2562–71.

61. Bekkering S, van den Munckhof I, Nielen T, Lamfers E, Dinarello C, Rutten J, et al. Innate immune cell activation and epigenetic remodeling in symptomatic and asymptomatic atherosclerosis in humans in vivo. Atherosclerosis. 2016;254:228–36.

62. Novakovic B, Habibi E, Wang SY, Arts RJW, Davar R, Megchelenbrink W, et al. Beta-glucan reverses the epigenetic state of LPS-induced immunological tolerance. Cell. 2016;167(5):1354–1368.e14.

63. Kleinnijenhuis J, Quintin J, Preijers F, Joosten LA, Ifrim DC, Saeed S, et al. Bacille Calmette-Guerin induces NOD2-dependent nonspecific protection from reinfection via epigenetic reprogramming of monocytes. Proc Natl Acad Sci U S A. 2012;109(43):17537–42.

64. Dos Santos JC, Barroso de Figueiredo AM, Teodoro Silva MV, Cirovic B, de Bree LCJ, Damen M, et al. beta-glucan-induced trained immunity protects against *Leishmania braziliensis* infection: a crucial role for IL-32. Cell Rep. 2019;28(10):2659–72.e6.

65. Cirovic B, de Bree LCJ, Groh L, Blok BA, Chan J, van der Velden W, et al. BCG vaccination in humans elicits trained immunity via the hematopoietic progenitor compartment. Cell Host Microbe. 2020;28(2):322–34.e5.

66. Mitroulis I, Ruppova K, Wang B, Chen LS, Grzybek M, Grinenko T, et al. Modulation of Myelopoiesis progenitors is an integral component of trained immunity. Cell. 2018;172(1–2):147–61.e12.

67. Hole CR, Wager CML, Castro-Lopez N, Campuzano A, Cai H, Wozniak KL, et al. Induction of memory-like dendritic cell responses in vivo. Nat Commun. 2019;10(1):2955.

68. Roquilly A, McWilliam HEG, Jacqueline C, Tian Z, Cinotti R, Rimbert M, et al. Local modulation of antigen-presenting cell development after resolution of pneumonia induces long-term susceptibility to secondary infections. Immunity. 2017;47(1):135–47.e5.

69. Datta M, Staszewski O, Raschi E, Frosch M, Hagemeyer N, Tay TL, et al. Histone deacetylases 1 and 2 regulate microglia function during development, homeostasis, and neurodegeneration in a context-dependent manner. Immunity. 2018;48(3):514–29.e6.

70. Wendeln AC, Degenhardt K, Kaurani L, Gertig M, Ulas T, Jain G, et al. Innate immune memory in the brain shapes neurological disease hallmarks. Nature. 2018;556(7701):332–8.

71. Schlums H, Cichocki F, Tesi B, Theorell J, Beziat V, Holmes TD, et al. Cytomegalovirus infection drives adaptive epigenetic diversification of NK cells with altered signaling and effector function. Immunity. 2015;42(3):443–56.

72. Rasid O, Chevalier C, Camarasa TM, Fitting C, Cavaillon JM, Hamon MA. H3K4me1 supports memory-like NK cells induced by systemic inflammation. Cell Rep. 2019;29(12):3933–45.e3.

73. Weizman OE, Song E, Adams NM, Hildreth AD, Riggan L, Krishna C, et al. Mouse cytomegalovirus-experienced ILC1s acquire a memory response dependent on the viral glycoprotein m12. Nat Immunol. 2019;20(8):1004–11.

74. Neeland MR, Andorf S, Manohar M, Dunham D, Lyu SC, Dang TD, et al. Mass cytometry reveals cellular fingerprint associated with IgE+ peanut tolerance and allergy in early life. Nat Commun. 2020;11(1):1091.

75. Wang T, Liu H, Lian G, Zhang SY, Wang X, Jiang C. HIF1α-induced glycolysis metabolism is essential to the activation of inflammatory macrophages. Mediators Inflamm. 2017;2017: 9029327.

76. Arts RJ, Novakovic B, Ter Horst R, Carvalho A, Bekkering S, Lachmandas E, et al. Glutaminolysis and fumarate accumulation integrate immunometabolic and epigenetic programs in trained immunity. Cell Metab. 2016;24(6):807–19.

77. Etchegaray JP, Mostoslavsky R. Interplay between metabolism and epigenetics: a nuclear adaptation to environmental changes. Mol Cell. 2016;62(5):695–711.

78. Stensballe LG, Nante E, Jensen IP, Kofoed PE, Poulsen A, Jensen H, et al. Acute lower respiratory tract infections and respiratory syncytial virus in infants in Guinea-Bissau: a beneficial effect of BCG vaccination for girls community based case-control study. Vaccine. 2005;23(10):1251–7.

79. Jensen KJ, Larsen N, Biering-Sorensen S, Andersen A, Eriksen HB, Monteiro I, et al. Heterologous immunological effects of early BCG vaccination in low-birth-weight infants in Guinea-Bissau: a randomized-controlled trial. J Infect Dis. 2015;211(6):956–67.

80. Nemes E, Geldenhuys H, Rozot V, Rutkowski KT, Ratangee F, Bilek N, et al. Prevention of *M. tuberculosis* infection with H4: IC31 vaccine or BCG revaccination. N Engl J Med. 2018; 379(2):138–49.

81. Giamarellos-Bourboulis EJ, Tsilika M, Moorlag S, Antonakos N, Kotsaki A, Domínguez-Andrés J, et al. Activate: randomized clinical trial of BCG vaccination against infection in the elderly. Cell. 2020;183(2):315–23.e9.

82. Ten Doesschate T, Moorlag S, van der Vaart TW, Taks E, Debisarun P, Ten Oever J, et al. Two Randomized Controlled Trials of Bacillus Calmette-Guerin Vaccination to reduce absenteeism among health care workers and hospital admission by elderly persons during the COVID-19 pandemic: a structured summary of the study protocols for two randomised controlled trials. Trials. 2020;21(1):481.

83. Katzmarski N, Domínguez-Andrés J, Cirovic B, Renieris G, Ciarlo E, Le Roy D, et al. Transmission of trained immunity and heterologous resistance to infections across generations. Nat Immunol. 2021;22(11):1382–90.

84. Berendsen M, Schaltz-Buchholzer F, Bles P, Biering-Sorensen S, Jensen KJ, Monteiro I, et al. Parental Bacillus Calmette-Guerin vaccine scars decrease infant mortality in the first six weeks of life: a retrospective cohort study. EClinicalMedicine. 2021;39:101049.

85. de Candia P, Matarese G. Reimagining an immunological dogma. Nat Immunol. 2021;22(11):1355–8.

86. Jiang Y, Liu Y, Lu H, Sun SC, Jin W, Wang X, et al. Epigenetic activation during T helper 17 cell differentiation is mediated by tripartite motif containing 28. Nat Commun. 2018;9(1):1424.

87. Dutta A, Venkataganesh H, Love PE. New insights into epigenetic regulation of T cell differentiation. Cell. 2021;10(12): 3459.

88. Leoni C, Vincenzetti L, Emming S, Monticelli S. Epigenetics of T lymphocytes in health and disease. Swiss Med Wkly. 2015; 145:w14191.

89. Zhang F, Boothby M. T helper type 1-specific Brg1 recruitment and remodeling of nucleosomes positioned at the IFN-gamma promoter are Stat4 dependent. J Exp Med. 2006;203(6): 1493–505.

90. Hwang ES, Szabo SJ, Schwartzberg PL, Glimcher LH. T helper cell fate specified by kinase-mediated interaction of T-bet with GATA-3. Science. 2005;307(5708):430–3.

91. Cote-Sierra J, Foucras G, Guo L, Chiodetti L, Young HA, Hu-Li J, et al. Interleukin 2 plays a central role in Th2 differentiation. Proc Natl Acad Sci U S A. 2004;101(11):3880–5.

92. Tumes DJ, Onodera A, Suzuki A, Shinoda K, Endo Y, Iwamura C, et al. The polycomb protein Ezh2 regulates differentiation and plasticity of CD4(+) T helper type 1 and type 2 cells. Immunity. 2013;39(5):819–32.

93. Allan RS, Zueva E, Cammas F, Schreiber HA, Masson V, Belz GT, et al. An epigenetic silencing pathway controlling T helper 2 cell lineage commitment. Nature. 2012;487(7406): 249–53.

94. Vahedi G, Takahashi H, Nakayamada S, Sun HW, Sartorelli V, Kanno Y, et al. STATs shape the active enhancer landscape of T cell populations. Cell. 2012;151(5):981–93.

95. Hasan M, Neumann B, Haupeltshofer S, Stahlke S, Fantini MC, Angstwurm K, et al. Activation of TGF-beta-induced non-Smad signaling pathways during Th17 differentiation. Immunol Cell Biol. 2015;93(7):662–72.

96. Durant L, Watford WT, Ramos HL, Laurence A, Vahedi G, Wei L, et al. Diverse targets of the transcription factor STAT3 contribute to T cell pathogenicity and homeostasis. Immunity. 2010;32(5):605–15.

97. Ivanov II, McKenzie BS, Zhou L, Tadokoro CE, Lepelley A, Lafaille JJ, et al. The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17+ T helper cells. Cell. 2006;126(6):1121–33.

98. Renaude E, Kroemer M, Loyon R, Binda D, Borg C, Guittaut M, et al. The fate of Th17 cells is shaped by epigenetic modifications and remodeled by the tumor microenvironment. Int J Mol Sci. 2020;21(5):1673.

99. Wei G, Wei L, Zhu J, Zang C, Hu-Li J, Yao Z, et al. Global mapping of H3K4me3 and H3K27me3 reveals specificity and plasticity in lineage fate determination of differentiating CD4+ T cells. Immunity. 2009;30(1):155–67.

100. Nakayamada S, Kanno Y, Takahashi H, Jankovic D, Lu KT, Johnson TA, et al. Early Th1 cell differentiation is marked by a Tfh cell-like transition. Immunity. 2011;35(6):919–31.

101. Ma X, Nakayamada S, Kubo S, Sakata K, Yamagata K, Miyazaki Y, et al. Expansion of T follicular helper-T helper 1 like cells through epigenetic regulation by signal transducer and activator of transcription factors. Ann Rheum Dis. 2018; 77(9):1354–61.

102. Powell MD, Read KA, Sreekumar BK, Jones DM, Oestreich KJ. IL-12 signaling drives the differentiation and function of a TH1-derived TFH1-like cell population. Sci Rep. 2019;9(1):13991.

103. Carpio VH, Aussenac F, Puebla-Clark L, Wilson KD, Villarino AV, Dent AL, et al. T helper plasticity is orchestrated by STAT3, Bcl6, and Blimp-1 balancing pathology and protection in malaria. iScience. 2020;23(7):101310.

104. Bauquet AT, Jin H, Paterson AM, Mitsdoerffer M, Ho IC, Sharpe AH, et al. The costimulatory molecule ICOS regulates the expression of c-Maf and IL-21 in the development of

follicular T helper cells and TH-17 cells. Nat Immunol. 2009; 10(2):167–75.

105. Stockinger B, Veldhoen M, Martin B. Th17 T cells: linking innate and adaptive immunity. Semin Immunol. 2007;19(6): 353–61.

106. Veldhoen M, Hocking RJ, Flavell RA, Stockinger B. Signals mediated by transforming growth factor-beta initiate autoimmune encephalomyelitis, but chronic inflammation is needed to sustain disease. Nat Immunol. 2006;7(11):1151–6.

107. Zhou L, Ivanov II, Spolski R, Min R, Shenderov K, Egawa T, et al. IL-6 programs T(H)-17 cell differentiation by promoting sequential engagement of the IL-21 and IL-23 pathways. Nat Immunol. 2007;8(9):967–74.

108. Langrish CL, Chen Y, Blumenschein WM, Mattson J, Basham B, Sedgwick JD, et al. IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. J Exp Med. 2005;201(2):233–40.

109. Kroenke MA, Eto D, Locci M, Cho M, Davidson T, Haddad EK, et al. Bcl6 and Maf cooperate to instruct human follicular helper CD4 T cell differentiation. J Immunol. 2012; 188(8):3734–44.

110. Andris F, Denanglaire S, Anciaux M, Hercor M, Hussein H, Leo O. The transcription factor c-Maf promotes the differentiation of follicular helper T cells. Front Immunol. 2017;8:480.

111. Josefowicz SZ, Lu LF, Rudensky AY. Regulatory T cells: mechanisms of differentiation and function. Annu Rev Immunol. 2012;30:531–64.

112. Ohkura N, Hamaguchi M, Morikawa H, Sugimura K, Tanaka A, Ito Y, et al. T cell receptor stimulation-induced epigenetic changes and Foxp3 expression are independent and complementary events required for Treg cell development. Immunity. 2012;37(5):785–99.

113. Zheng Y, Josefowicz S, Chaudhry A, Peng XP, Forbush K, Rudensky AY. Role of conserved non-coding DNA elements in the Foxp3 gene in regulatory T-cell fate. Nature. 2010; 463(7282):808–12.

114. Placek K, Hu G, Cui K, Zhang D, Ding Y, Lee JE, et al. MLL4 prepares the enhancer landscape for Foxp3 induction via chromatin looping. Nat Immunol. 2017;18(9):1035–45.

115. Kawakami R, Kitagawa Y, Chen KY, Arai M, Ohara D, Nakamura Y, et al. Distinct Foxp3 enhancer elements coordinate development, maintenance, and function of regulatory T cells. Immunity. 2021;54(5):947–61. e8.

116. Zemmour D, Pratama A, Loughhead SM, Mathis D, Benoist C. Flicr, a long noncoding RNA, modulates Foxp3 expression and autoimmunity. Proc Natl Acad Sci U S A. 2017;114(17): E3472–E80.

117. De Rosa V, Galgani M, Porcellini A, Colamatteo A, Santopaolo M, Zuchegna C, et al. Glycolysis controls the induction of human regulatory T cells by modulating the expression of FOXP3 exon 2 splicing variants. Nat Immunol. 2015;16(11):1174–84.

118. Helmin KA, Morales-Nebreda L, Torres Acosta MA, Anekalla KR, Chen SY, Abdala-Valencia H, et al. Maintenance DNA methylation is essential for regulatory T cell development and stability of suppressive function. J Clin Invest. 2020; 130(12):6571–87.

119. Xie X, Stubbington MJ, Nissen JK, Andersen KG, Hebenstreit D, Teichmann SA, et al. The regulatory T cell

lineage factor Foxp3 regulates gene expression through several distinct mechanisms mostly independent of direct DNA binding. PLoS Genet. 2015;11(6):e1005251.

120. Mailer RK, Falk K, Rotzschke O. Absence of leucine zipper in the natural FOXP3Delta2Delta7 isoform does not affect dimerization but abrogates suppressive capacity. PLoS One. 2009; 4(7):e6104.

121. Joly AL, Liu S, Dahlberg CI, Mailer RK, Westerberg LS, Andersson J. Foxp3 lacking exons 2 and 7 is unable to confer suppressive ability to regulatory T cells in vivo. J Autoimmun. 2015;63:23–30.

122. Kaur G, Goodall JC, Jarvis LB, Hill Gaston JS. Characterisation of Foxp3 splice variants in human CD4+ and CD8+ T cells—identification of Foxp3Delta7 in human regulatory T cells. Mol Immunol. 2010;48(1–3):321–32.

123. Li C, Jiang S, Liu SQ, Lykken E, Zhao LT, Sevilla J, et al. MeCP2 enforces Foxp3 expression to promote regulatory T cells' resilience to inflammation. Proc Natl Acad Sci U S A. 2014;111(27):E2807–16.

124. Ladle BH, Li KP, Phillips MJ, Pucsek AB, Haile A, Powell JD, et al. De novo DNA methylation by DNA methyltransferase 3a controls early effector CD8+ T-cell fate decisions following activation. Proc Natl Acad Sci U S A. 2016;113(38):10631–6.

125. Verbaro DJ, Sakurai N, Kim B, Shinkai Y, Egawa T. Cutting edge: the histone methyltransferase G9a is required for silencing of helper T lineage-associated genes in proliferating CD8 T cells. J Immunol. 2018;200(12):3891–6.

126. Tsao HW, Kaminski J, Kurachi M, Barnitz RA, DiIorio MA, LaFleur MW, et al. Batf-mediated epigenetic control of effector CD8(+) T cell differentiation. Sci Immunol. 2022;7(68): eabi4919.

127. Firestein R, Cui X, Huie P, Cleary ML. Set domain-dependent regulation of transcriptional silencing and growth control by SUV39H1, a mammalian ortholog of drosophila Su(var)3-9. Mol Cell Biol. 2000;20(13):4900–9.

128. Pace L, Goudot C, Zueva E, Gueguen P, Burgdorf N, Waterfall JJ, et al. The epigenetic control of stemness in CD8 (+) T cell fate commitment. Science. 2018;359(6372):177–86.

129. Sung BY, Lin YH, Kong Q, Shah PD, Glick Bieler J, Palmer S, et al. Wnt activation promotes memory T cell polyfunctionality via epigenetic regulator PRMT1. J Clin Invest. 2022;132(2): e140508.

130. Shaknovich R, Cerchietti L, Tsikitas L, Kormaksson M, De S, Figueroa ME, et al. DNA methyltransferase 1 and DNA methylation patterning contribute to germinal center B-cell differentiation. Blood. 2011;118(13):3559–69.

131. Cobaleda C, Schebesta A, Delogu A, Busslinger M. Pax5: the guardian of B cell identity and function. Nat Immunol. 2007; 8(5):463–70.

132. Xu Z, Zan H, Pone EJ, Mai T, Casali P. Immunoglobulin class-switch DNA recombination: induction, targeting and beyond. Nat Rev Immunol. 2012;12(7):517–31.

133. Dominguez PM, Teater M, Chambwe N, Kormaksson M, Redmond D, Ishii J, et al. DNA methylation dynamics of germinal center B cells are mediated by AID. Cell Rep. 2015; 12(12):2086–98.

134. Loeffler M, Kreuz M, Haake A, Hasenclever D, Trautmann H, Arnold C, et al. Genomic and epigenomic co-evolution in follicular lymphomas. Leukemia. 2015;29(2):456–63.

135. Zan H, Casali P. Epigenetics of peripheral B-cell differentiation and the antibody response. Front Immunol. 2015;6:631.

136. Shi W, Liao Y, Willis SN, Taubenheim N, Inouye M, Tarlinton DM, et al. Transcriptional profiling of mouse B cell terminal differentiation defines a signature for antibody-secreting plasma cells. Nat Immunol. 2015;16(6):663–73.

137. Heinz S, Benner C, Spann N, Bertolino E, Lin YC, Laslo P, et al. Simple combinations of lineage-determining transcription factors prime cis-regulatory elements required for macrophage and B cell identities. Mol Cell. 2010;38(4):576–89.

138. Tellier J, Shi W, Minnich M, Liao Y, Crawford S, Smyth GK, et al. Blimp-1 controls plasma cell function through the regulation of immunoglobulin secretion and the unfolded protein response. Nat Immunol. 2016;17(3):323–30.

139. Yu J, Angelin-Duclos C, Greenwood J, Liao J, Calame K. Transcriptional repression by blimp-1 (PRDI-BF1) involves recruitment of histone deacetylase. Mol Cell Biol. 2000;20(7):2592–603.

140. Keller AD, Maniatis T. Only two of the five zinc fingers of the eukaryotic transcriptional repressor PRDI-BF1 are required for sequence-specific DNA binding. Mol Cell Biol. 1992;12(5):1940–9.

141. Ren B, Chee KJ, Kim TH, Maniatis T. PRDI-BF1/Blimp-1 repression is mediated by corepressors of the Groucho family of proteins. Genes Dev. 1999;13(1):125–37.

142. Nutt SL, Hodgkin PD, Tarlinton DM, Corcoran LM. The generation of antibody-secreting plasma cells. Nat Rev Immunol. 2015;15(3):160–71.

143. Shapiro-Shelef M, Calame K. Regulation of plasma-cell development. Nat Rev Immunol. 2005;5(3):230–42.

144. Gyory I, Wu J, Fejer G, Seto E, Wright KL. PRDI-BF1 recruits the histone H3 methyltransferase G9a in transcriptional silencing. Nat Immunol. 2004;5(3):299–308.

145. Li G, Zan H, Xu Z, Casali P. Epigenetics of the antibody response. Trends Immunol. 2013;34(9):460–70.

146. Baxter J, Sauer S, Peters A, John R, Williams R, Caparros ML, et al. Histone hypomethylation is an indicator of epigenetic plasticity in quiescent lymphocytes. EMBO J. 2004;23(22):4462–72.

147. Caganova M, Carrisi C, Varano G, Mainoldi F, Zanardi F, Germain PL, et al. Germinal center dysregulation by histone methyltransferase EZH2 promotes lymphomagenesis. J Clin Invest. 2013;123(12):5009–22.

148. Javierre BM, Fernandez AF, Richter J, Al-Shahrour F, Martin-Subero JI, Rodriguez-Ubreva J, et al. Changes in the pattern of DNA methylation associate with twin discordance in systemic lupus erythematosus. Genome Res. 2010;20(2):170–9.

149. Stagi S, Gulino AV, Lapi E, Rigante D. Epigenetic control of the immune system: a lesson from Kabuki syndrome. Immunol Res. 2016;64(2):345–59.

150. Placek K, Schultze JL, Aschenbrenner AC. Epigenetic reprogramming of immune cells in injury, repair, and resolution. J Clin Invest. 2019;129(8):2994–3005.

151. Mastrandrea LD. An overview of organ-specific autoimmune diseases including immunotherapy. Immunol Invest. 2015;44(8):803–16.

152. Lesage S, Goodnow CC. Organ-specific autoimmune disease: a deficiency of tolerogenic stimulation. J Exp Med. 2001;194(5):F31–6.

153. Wang YI, McDuffie M, Lafferty KJ. Antigen presentation and effector mechanisms in 'auto-immune' diabetes. J Gastroenterol Hepatol. 1991;6(6):574–9.

154. Balazs C. Hashimoto's thyroiditis, the model of organ-specific autoimmune disorders. Orv Hetil. 2007;148(Suppl 1):31–3.

155. de Lera AR, Ganesan A. Epigenetic polypharmacology: from combination therapy to multitargeted drugs. Clin Epigenetics. 2016;8:105.

156. Montalvo-Casimiro M, Gonzalez-Barrios R, Meraz-Rodriguez MA, Juarez-Gonzalez VT, Arriaga-Canon C, Herrera LA. Epidrug repurposing: discovering new faces of old acquaintances in cancer therapy. Front Oncol. 2020;10:605386.

157. Hu N, Qiu X, Luo Y, Yuan J, Li Y, Lei W, et al. Abnormal histone modification patterns in lupus CD4+ T cells. J Rheumatol. 2008;35(5):804–10.

158. Richardson B. Primer: epigenetics of autoimmunity. Nat Clin Pract Rheumatol. 2007;3(9):521–7.

159. Quddus J, Johnson KJ, Gavalchin J, Amento EP, Chrisp CE, Yung RL, et al. Treating activated CD4+ T cells with either of two distinct DNA methyltransferase inhibitors, 5-azacytidine or procainamide, is sufficient to cause a lupus-like disease in syngeneic mice. J Clin Invest. 1993;92(1):38–53.

160. Richardson B. Effect of an inhibitor of DNA methylation on T cells. II. 5-Azacytidine induces self-reactivity in antigen-specific T4+ cells. Hum Immunol. 1986;17(4):456–70.

161. Richardson BC, Liebling MR, Hudson JL. CD4+ cells treated with DNA methylation inhibitors induce autologous B cell differentiation. Clin Immunol Immunopathol. 1990;55(3):368–81.

162. Tsou PS, Coit P, Kilian NC, Sawalha AH. EZH2 modulates the DNA methylome and controls T cell adhesion through junctional adhesion molecule a in lupus patients. Arthritis Rheumatol. 2018;70(1):98–108.

163. Garcia BA, Busby SA, Shabanowitz J, Hunt DF, Mishra N. Resetting the epigenetic histone code in the MRL-lpr/lpr mouse model of lupus by histone deacetylase inhibition. J Proteome Res. 2005;4(6):2032–42.

164. Huber LC, Brock M, Hemmatazad H, Giger OT, Moritz F, Trenkmann M, et al. Histone deacetylase/acetylase activity in total synovial tissue derived from rheumatoid arthritis and osteoarthritis patients. Arthritis Rheum. 2007;56(4):1087–93.

165. Manabe H, Nasu Y, Komiyama T, Furumatsu T, Kitamura A, Miyazawa S, et al. Inhibition of histone deacetylase downregulates the expression of hypoxia-induced vascular endothelial growth factor by rheumatoid synovial fibroblasts. Inflamm Res. 2008;57(1):4–10.

166. Ahmed S, Riegsecker S, Beamer M, Rahman A, Bellini JV, Bhansali P, et al. Largazole, a class I histone deacetylase inhibitor, enhances TNF-alpha-induced ICAM-1 and VCAM-1 expression in rheumatoid arthritis synovial fibroblasts. Toxicol Appl Pharmacol. 2013;270(2):87–96.

167. Vojinovic J, Damjanov N, D'Urzo C, Furlan A, Susic G, Pasic S, et al. Safety and efficacy of an oral histone deacetylase inhibitor in systemic-onset juvenile idiopathic arthritis. Arthritis Rheum. 2011;63(5):1452–8.

168. Lei W, Luo Y, Lei W, Luo Y, Yan K, Zhao S, et al. Abnormal DNA methylation in CD4+ T cells from patients with systemic lupus erythematosus, systemic sclerosis, and dermatomyositis. Scand J Rheumatol. 2009;38(5):369–74.

169. Wang Y, Shu Y, Xiao Y, Wang Q, Kanekura T, Li Y, et al. Hypomethylation and overexpression of ITGAL (CD11a) in CD4 (+) T cells in systemic sclerosis. Clin Epigenetics. 2014;6(1):25.

170. Jiang H, Xiao R, Lian X, Kanekura T, Luo Y, Yin Y, et al. Demethylation of TNFSF7 contributes to CD70 overexpression in CD4+ T cells from patients with systemic sclerosis. Clin Immunol. 2012;143(1):39–44.

171. Ding W, Pu W, Wang L, Jiang S, Zhou X, Tu W, et al. Genome-wide DNA methylation analysis in systemic sclerosis reveals hypomethylation of IFN-associated genes in CD4(+) and CD8(+) T cells. J Invest Dermatol. 2018;138(5):1069–77.

172. Wang Y, Fan PS, Kahaleh B. Association between enhanced type I collagen expression and epigenetic repression of the FLI1 gene in scleroderma fibroblasts. Arthritis Rheum. 2006;54(7):2271–9.

173. Wang Y, Yang Y, Luo Y, Yin Y, Wang Q, Li Y, et al. Aberrant histone modification in peripheral blood B cells from patients with systemic sclerosis. Clin Immunol. 2013;149(1):46–54.

174. Hemmatazad H, Rodrigues HM, Maurer B, Brentano F, Pileckyte M, Distler JH, et al. Histone deacetylase 7, a potential target for the antifibrotic treatment of systemic sclerosis. Arthritis Rheum. 2009;60(5):1519–29.

175. Akhmetshina A, Palumbo K, Dees C, Bergmann C, Venalis P, Zerr P, et al. Activation of canonical Wnt signalling is required for TGF-beta-mediated fibrosis. Nat Commun. 2012;3:735.

176. Svegliati S, Marrone G, Pezone A, Spadoni T, Grieco A, Moroncini G, et al. Oxidative DNA damage induces the ATM-mediated transcriptional suppression of the Wnt inhibitor WIF-1 in systemic sclerosis and fibrosis. Sci Signal. 2014;7(341):ra84.

177. Dees C, Schlottmann I, Funke R, Distler A, Palumbo-Zerr K, Zerr P, et al. The Wnt antagonists DKK1 and SFRP1 are downregulated by promoter hypermethylation in systemic sclerosis. Ann Rheum Dis. 2014;73(6):1232–9.

178. Teruel M, Barturen G, Martinez-Bueno M, Castellini-Perez O, Barroso-Gil M, Povedano E, et al. Integrative epigenomics in Sjogren s syndrome reveals novel pathways and a strong interaction between the HLA, autoantibodies and the interferon signature. Sci Rep. 2021;11(1):23292.

179. Yu X, Liang G, Yin H, Ngalamika O, Li F, Zhao M, et al. DNA hypermethylation leads to lower FOXP3 expression in CD4+ T cells of patients with primary Sjogren's syndrome. Clin Immunol. 2013;148(2):254–7.

180. Graves MC, Benton M, Lea RA, Boyle M, Tajouri L, Macartney-Coxson D, et al. Methylation differences at the HLA-DRB1 locus in CD4+ T-cells are associated with multiple sclerosis. Mult Scler. 2014;20(8):1033–41.

181. Sokratous M, Dardiotis E, Tsouris Z, Bellou E, Michalopoulou A, Siokas V, et al. Deciphering the role of DNA methylation in multiple sclerosis: emerging issues. Auto Immun Highlights. 2016;7(1):12.

182. Janson PC, Linton LB, Bergman EA, Marits P, Eberhardson M, Piehl F, et al. Profiling of CD4+ T cells with epigenetic immune lineage analysis. J Immunol. 2011;186(1):92–102.

183. Noori-Zadeh A, Mesbah-Namin SA, Saboor-Yaraghi AA. Epigenetic and gene expression alterations of FOXP3 in the T cells of EAE mouse model of multiple sclerosis. J Neurol Sci. 2017; 375:203–8.

184. Arakawa Y, Watanabe M, Inoue N, Sarumaru M, Hidaka Y, Iwatani Y. Association of polymorphisms in DNMT1, DNMT3A, DNMT3B, MTHFR and MTRR genes with global DNA methylation levels and prognosis of autoimmune thyroid disease. Clin Exp Immunol. 2012;170(2):194–201.

185. Limbach M, Saare M, Tserel L, Kisand K, Eglit T, Sauer S, et al. Epigenetic profiling in CD4+ and CD8+ T cells from Graves' disease patients reveals changes in genes associated with T cell receptor signaling. J Autoimmun. 2016;67:46–56.

186. Li Y, Zhao M, Hou C, Liang G, Yang L, Tan Y, et al. Abnormal DNA methylation in CD4+ T cells from people with latent autoimmune diabetes in adults. Diabetes Res Clin Pract. 2011; 94(2):242–8.

187. Miao F, Smith DD, Zhang L, Min A, Feng W, Natarajan R. Lymphocytes from patients with type 1 diabetes display a distinct profile of chromatin histone H3 lysine 9 dimethylation: an epigenetic study in diabetes. Diabetes. 2008;57(12):3189–98.

188. Jayaraman S, Arianas M, Jayaraman A. Epigenetic modulation of selected immune response genes and altered functions of T lymphocytes and macrophages collectively contribute to autoimmune diabetes protection. Biochimica et Biophysica Acta Adv. 2021;1:100031.

189. Farh KK, Marson A, Zhu J, Kleinewietfeld M, Housley WJ, Beik S, et al. Genetic and epigenetic fine mapping of causal autoimmune disease variants. Nature. 2015;518(7539):337–43.

190. Mumbach MR, Satpathy AT, Boyle EA, Dai C, Gowen BG, Cho SW, et al. Enhancer connectome in primary human cells identifies target genes of disease-associated DNA elements. Nat Genet. 2017;49(11):1602–12.

191. Lu Q, Kaplan M, Ray D, Ray D, Zacharek S, Gutsch D, et al. Demethylation of ITGAL (CD11a) regulatory sequences in systemic lupus erythematosus. Arthritis Rheum. 2002;46(5): 1282–91.

192. Oelke K, Lu Q, Richardson D, Wu A, Deng C, Hanash S, et al. Overexpression of CD70 and overstimulation of IgG synthesis by lupus T cells and T cells treated with DNA methylation inhibitors. Arthritis Rheum. 2004;50(6):1850–60.

193. Marks PA, Dokmanovic M. Histone deacetylase inhibitors: discovery and development as anticancer agents. Expert Opin Investig Drugs. 2005;14(12):1497–511.

194. Fang TJ, Lin YZ, Liu CC, Lin CH, Li RN, Wu CC, et al. Methylation and gene expression of histone deacetylases 6 in systemic lupus erythematosus. Int J Rheum Dis. 2016;19(10):968–73.

195. Horiuchi M, Morinobu A, Chin T, Sakai Y, Kurosaka M, Kumagai S. Expression and function of histone deacetylases in rheumatoid arthritis synovial fibroblasts. J Rheumatol. 2009; 36(8):1580–9.

196. Konsta OD, Charras A, Le Dantec C, Kapsogeorgeou E, Bordron A, Brooks WH, et al. Epigenetic modifications in salivary glands from patients with Sjogren's syndrome affect cytokeratin 19 expression. Bull Group Int Rech Sci Stomatol Odontol. 2016;53(1):e01.