REVIEW

doi: 10.1111/sji.12589

Stochastics of Cellular Differentiation Explained by Epigenetics: The Case of T-Cell Differentiation and Functional Plasticity

J. Bhat*, J. Helmuth†, G. Chitadze*, L. Kouakanou*, C. Peters*, M. Vingron‡, O. Ammerpohl§ & D. Kabelitz*

*Institute of Immunology, University of Kiel and University Hospital Schleswig-Holstein Campus Kiel, Kiel, Germany; †Otto-Warburg-Laboratories: Epigenomics, Max Planck Institute for Molecular Genetics, Berlin, Germany; ‡Department of Computational Molecular Biology, Max Planck Institute for Molecular Genetics, Berlin, Germany; and §Institute of Human Genetics, University of Kiel and University Hospital Schleswig-Holstein Campus Kiel, Kiel, Germany

Received 1 July 2017; Accepted in revised form 6 August 2017

Correspondence to: Dieter Kabelitz, Institute of Immunology, University of Kiel, Arnold-Heller-Str.3, Bldg. 17, D-24105 Kiel, Germany. E-mail: dietrich.kabelitz@uksh.de

Abstract

Epigenetic marks including histone modifications and DNA methylation are associated with the regulation of gene expression and activity. In addition, an increasing number of non-coding RNAs with regulatory activity on gene expression have been identified. Alongside, technological advancements allow for the analysis of these mechanisms with high resolution up to the single-cell level. For instance, the assay for transposase-accessible chromatin using sequencing (ATAC-seq) simultaneously probes for chromatin accessibility and nucleosome positioning. Thus, it provides information on two levels of epigenetic regulation. Development and differentiation of T cells into functional subset cells including memory T cells are dynamic processes driven by environmental signals. Here, we briefly review the current knowledge of how epigenetic regulation contributes to subset specification, differentiation and memory development in T cells. Specifically, we focus on epigenetic mechanisms differentially active in the two distinct T cell populations expressing $\alpha\beta$ or $\gamma\delta$ T cell receptors. We also discuss examples of epigenetic alterations of T cells in autoimmune diseases. DNA methylation and histone acetylation are subject to modification by several classes of 'epigenetic modifiers', some of which are in clinical use or in preclinical development. Therefore, we address the impact of some epigenetic modifiers on T-cell activation and differentiation, and discuss possible synergies with T cell-based immunotherapeutic strategies.

Epigenetic mechanisms and technological advancements

Epigenetic mechanisms are associated with gene expression and ultimately protein expression. The chromatin and RNA molecules are key players in this complex network. Core structures of the chromatin are DNA–protein complexes called nucleosomes, which consist of 147 bp of DNA and a heterodimer of histones H1, H2, H3 and H4. The nucleosomes can act as a barrier to DNA-binding proteins, but processes such as transcription and replication require free access to the DNA template. The nucleosome remodelling involves changes in DNA–protein interactions by disrupting, disassembling or moving nucleosomes and thus allowing DNA to get accessible for further processes. Consequently, chromatin accessibility and nucleosome remodelling are highly important for gene expression [1]. The chromatin is substantially modulated by epigenetic modifications, notably DNA and histone modifications. DNA modifications include the most widely studied 5-methylcytosine (5mC) and the relatively newly discovered 5-hydroxymethylation (5hmC), 5-formylcytosine (5fC) and 5-carboxycytosine (5caC) [2, 3]. BeadChip arrays are commonly used for analysis of DNA methylation. Compared to sequencing methods like whole-genome bisulphite sequencing (WGBS) or reduced-representation bisulphite sequencing (RRBS), which need extensive bioinformatics expertise, array-based genome-wide studies are easy to handle but cover only a fraction of all CpG sites. Importantly, a substantial proportion of differentially methylated regions (DMRs) are located outside the CpG islands in the 'open sea', yet are important for regulation of gene expression. Gene expression is further regulated at the epigenetic level through histone modifications including acetylation and (tri)methylation. Alternatively, regulation of gene expression targeting non-histone proteins can be mediated by competitive non-histone-like modifications in a non-epigenetic process [4]. An interrelated role of both

© 2017 The Foundation for the Scandinavian Journal of Immunology

SCANDINAVIAN JOURNAL OF Immunology AN INTERNATIONAL MONTHLY JOURNAL

epigenetic and non-epigenetic processes in the regulation of inflammatory genes is addressed through the development of synthetic compounds targeting proteins that recognize post-translationally modified histones [5]. However, this review will focus on the epigenetic processes.

Once a particular gene is expressed, RNA transcripts are produced. At this stage, RNA undergoes several ways of controlled regulation. Together with protein-coding transcripts (mRNA), non-coding RNA (ncRNA) with a size between 20 and 200 nucleotides, for example microRNA/miRNA, small nucleolar RNA/snoRNA, small nuclear RNA/snRNA, piwi-interacting RNA/piRNA, transfer RNA/tRNA, and long non-coding RNA with more than 200 nucleotides, for example antisense ncRNA, enhancer ncRNA/eRNA, intergenic RNA/lincRNA, pseudogene RNA, 3′ UTR ncRNA, play important roles in the overall regulation of RNA expression [6, 7]. Last but not least, the successfully translated protein undergoes various post-translational modifications such as palmitoylation, phosphorylation and ADP-ribosylation with significance for cellular differentiation and cell signalling [8].

Recently, innovative technologies became available to study transcription and epigenetic regulation like histone modifications and chromatin accessibility on a genome-wide level. High-throughput RNA-based sequencing (RNA-seq) is used for global transcriptome analysis up to the single-cell level [9] and can be also applied to non-coding RNAs [10, 11]. Histones can be modified by methylation or acetylation at specific amino acids which can be studied by chromatin immunoprecipitation followed by deep sequencing (ChIP-seq) [12]. Such active epigenetic 'marks' are for instance the trimethylation of histone H3 at Lysine 4 (H3K4me3) or acetylation of histone H3 at Lysine 27 (H3K27ac) associated with actively transcribed genes, whereas trimethylation of histone H3 at Lysine 27 (H3K27me3) is associated with repressed transcription [13]. To study chromatin accessibility, the assay for transposase-accessible chromatin with high-throughput sequencing (ATAC-seq) was developed. It is a powerful method for the simultaneous analysis of chromatin accessibility, nucleosome positioning and transcription factor occupancy [14]. Again, this method has been extended to the single-cell level [15], and further refinements now allow the combination with visualization where the accessible genome can be imaged *in situ* ('ATAC-see') and the corresponding cells are accessible to cell sorting [16]. A summary of current approaches as applied to the epigenetic analysis of T cells is given in Table 1. Evidently, all these approaches generate huge data sets, particularly when applied to complex research questions such as the differentiation of T cells from naïve *via* effector to memory cells during an ongoing immune response. Therefore, appropriate bioinformatics resources are mandatory, and novel algorithms and platforms have been developed to integrate the analysis of multiple approaches [17, 18]. A schematic overview of the principles of epigenetic mechanisms of gene regulation is presented in Fig. 1.

Table 1 Novel technical approaches to study epigenetic processes in T cells. A summary of representative studies on T cells and their functions with a focus on respective epigenetic processes.

Epigenetic process	Techniques	T-cell subset(s)	References
Chromatin accessibility	ATAC-seq	CD4, CD8	[14, 110]
	MNase-seq	CD4	[111]
	DNase-seq	CD4	[112]
Nucleosome positioning	ATAC-seq	CD4	[14]
	NOMe-seq	CD4	[71]
	MNase-seq	CD4	[111]
Transcription factor occupancy	ATAC-seq	CD4	[14]
	DNase -seq	CD4	[112]
Enhancer activity quantification	CapStarr-seq	$CD4^+CD8^+$	[113]
Histone modifications	ChIP-seq	CD4, $\gamma\delta$	[60, 114]
DNA methylation	450k arrays	CD4, CD8	[21]
	EPIC arrays	–	–
	NOMe-seq	CD4	[71]
	WGBS	CD4, CD8	[115]
	MeDIP-seq	CD4, CD8	[21]
DNA hydroxymethylation	CMS-IP-coupled sequencing	CD4, CD8	[52]
RNA expression	Gene arrays	$\gamma\delta$, $\alpha\beta$	[56]
	RNA-seq	CD4, Treg	[116, 117]
Polymerase pausing	GRO-seq	CD4	[118]

ATAC-seq, assay for transposase-accessible chromatin with high-throughput sequencing; MNase-seq, micrococcal nuclease digestion combined with sequencing; DNase-seq, DNase I digestion combined with high-throughput sequencing; NOMe-seq, nucleosome occupancy and methylome sequencing; CapStarr-seq, self-transcribing active regulatory region sequencing using capture approach; ChIP-seq, chromatin immunoprecipitation assay with sequencing; WGBS, whole-genome bisulphite sequencing; MeDIP-seq, methylated DNA immunoprecipitation assay with sequencing; CMS-IP, cytosine-5-methylenesulphonate immunoprecipitation; GRO-seq, global run-on sequencing.

Epigenetics of T-cell specification

T cells are generated from haematopoietic stem cells and undergo T-cell receptor (TCR) repertoire development and fitness selection ('central tolerance') during intrathymic differentiation. The cell fate decision of haematopoietic stem cells and their differentiation into distinct cell lineages is governed by epigenetic processes including directional changes in DNA methylation [19] (see Fig. 2 for an overview). Recent studies have investigated the specification of T cells as compared to other leukocytes on a global (epi)genetic level. Lara-Astiaso and coworkers focused on the chromatin state dynamics in 16 well-defined cell populations during haematopoiesis spanning from multipotent progenitor stages to common lineage (myeloid, lymphoid) progenitors and terminally differentiated cells including CD4 and CD8 T cells. To achieve this with only a few cells, they developed an indexing-first

© 2017 The Foundation for the Scandinavian Journal of Immunology

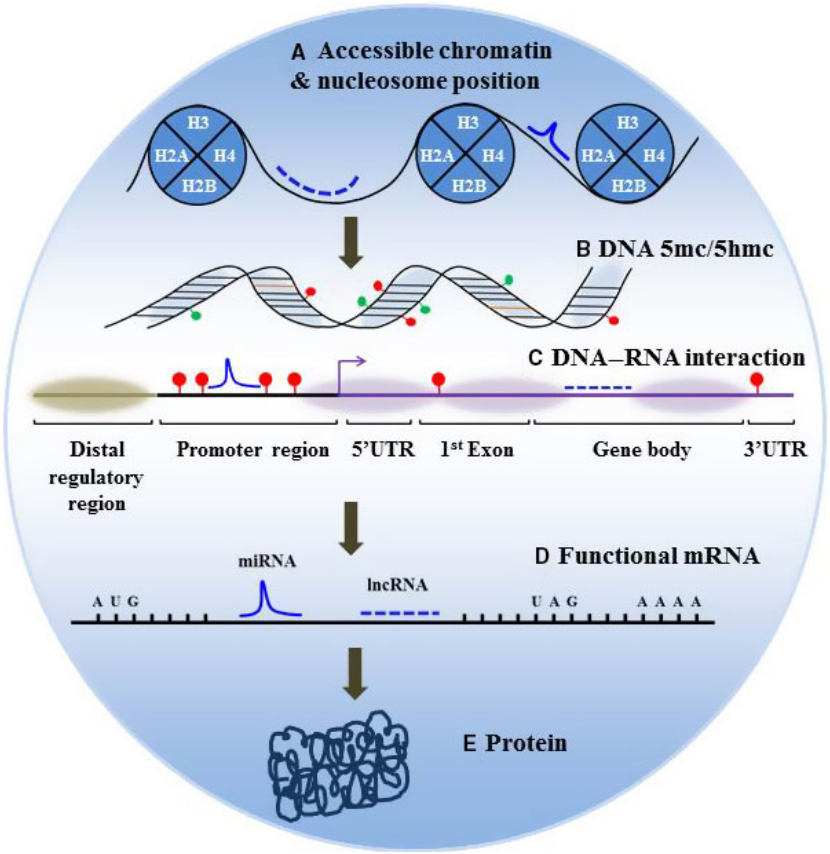

Figure 1 A schematic overview of epigenetic mechanisms and their functional role. Gene expression is regulated at multiple levels and is mediated by a multitude of molecular mechanisms. (A) Chromatin accessibility and nucleosome positioning are primarily important, as this facilitates the binding of transcription factors and associated machinery to regulatory elements. (B) The presence of DNA modifications such as methylation (5mc) and 5-hydroxymethylation (5hmc) on cytosine forms the basis of gene expression or silencing. (C) The interaction between DNA and RNA regulates gene expression depending on the genomic region of interaction. (D) An important class of epigenetic modifications based on regulatory RNA subspecies such as microRNAs (miRNA) and long non-coding RNAs (lncRNA) mainly determines the fate of RNA expression. (E) The protein translated from functional mRNA undergoes a number of post-translational modifications (PTMs). 5hmc and 5mc are represented by red and green marks on the DNA, respectively. miRNAs (lncRNAs) are indicated by the solid (dashed) blue lines.

chromatin IP (iChIP) method where barcoding of the total cellular chromatin from sorted cell populations is performed, thus enabling multiple chromatin-barcoded samples to be pooled for ChIP in one well [20]. They profiled four histone marks in H3 to define promoter (high H3K4me3) and enhancer (high H3K4me1/2 and low H3K4me3) regions and enhancer activity by combined H3K27ac and RNA expression levels. Thereby, Lara-Astiaso and coworkers confirmed previously described lineage-specific enhancers, but additionally identified a number of enhancers which are established *de novo* during haematopoiesis. This study revealed that lineage-specific enhancers are associated with transcription factor cohorts, confirming the notion that the transcription factors PU.1, GATA1 and Foxo1 are potential regulators of myeloid, erythroid and lymphoid enhancers, respectively [20]. Genome-wide methylation analyses performed by Zilbauer *et al.* in a small group of healthy individuals described cell type-specific hypomethylated regions correlating with gene transcription in CD4 and CD8 T cells in comparison with purified B cells, neutrophils and monocytes [21]. Focusing on the interindividual genomewide transcriptional and epigenetic specification of human immune cells in large cohorts of healthy individuals, two recent studies have further illustrated in detail how adaptive T cells segregate from innate monocytes and neutrophils [22, 23]. Not unexpectedly, characteristic epigenomic alterations have been attributed to the ageing process, for example large numbers of DMRs have been identified in CD4 T cells from three cohorts of newborns, middle-aged and long-lived (centenarian) individuals which were correlated to transcriptional regulation [24, 25]. Genomewide methylation studies were also performed in CD4 T cell subsets which specifically differ between young and old individuals, for example based on CD28 expression. CD28 delivers an essential costimulatory signal during T-cell activation, and there is an age-dependent decline in CD28 expression as part of the immunosenescence process [26]. Suarez-Álvarez and colleagues studied genome-wide DNA methylation and gene expression profiles of CD28-negative and CD28-positive CD4 T cells and identified hundreds of differentially methylated genes related to chemokine/cytokine signalling, cytotoxic activity and TCR signalling, in line with pro-inflammatory features of CD28-negative CD4 T cells [27]. Other investigators characterized naïve CD8 T cells from young and old individuals; the integrated analysis of ATAC-seq and RNA-seq data again helped to identify age-related transcription networks and revealed a loss of chromatin accessibility in aged CD8 T cells [28]. Another recent publication reported on the epigenetic landscape of human neonatal CD8 T cells in comparison with adult healthy donors. By combined

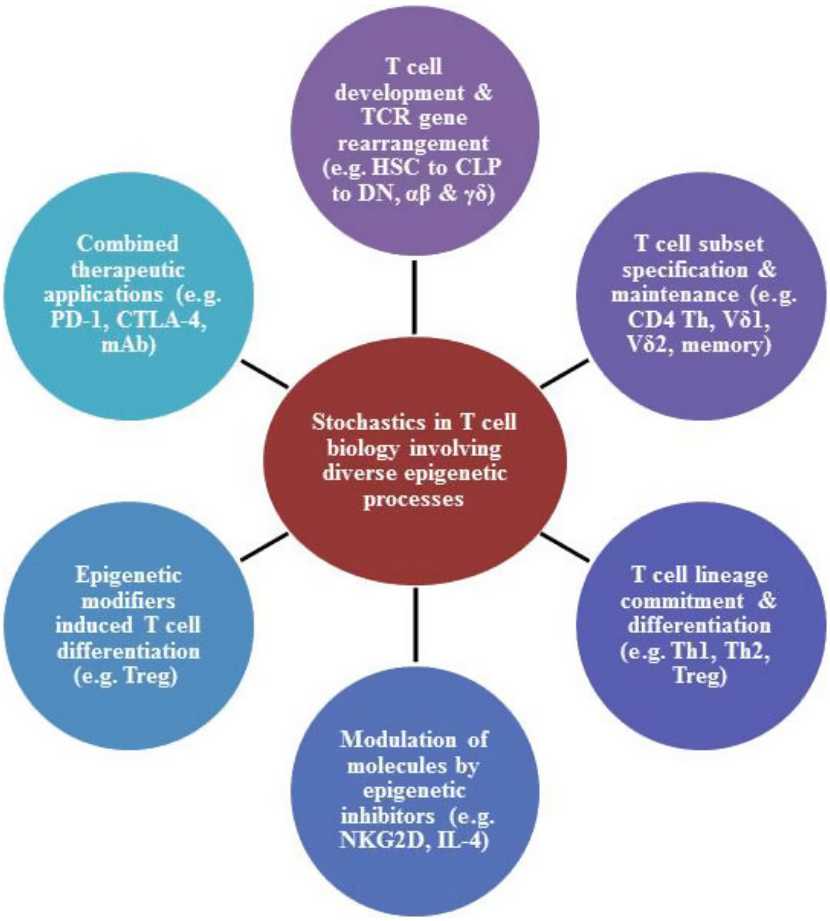

Figure 2 The functional significance of epigenetic processes in T-cell biology. Various epigenetic mechanisms alone or in combination lead to distinct functional responses of T cells, T-cell development and differentiation. These functional processes of T cells can be further modulated by epigenetic modifiers. An understanding of distinct epigenetic processes has translational perspectives and may lead to clinical applications in combination with other therapeutic approaches. The representative example(s) are mentioned in parentheses. TCR, T-cell receptor; HSC, haematopoietic stem cells; CLP, common lymphoid progenitor; DN, double negative; Th, helper T cells; Treg, regulatory T cells; NKG2D, natural killer group 2D; IL-4, interleukin-4; PD-1, programmed cell death protein 1; CTLA-4, cytotoxic T lymphocyte-associated protein 4; mAb, monoclonal antibody.

ChIP-seq and RNA-seq, these authors found that neonate CD8 T cells are skewed towards innate immune responses with low cytotoxic potential [29].

Depending on the priming conditions, mature T cells can differentiate into functionally distinct subsets, notably Th1, Th2, Th17, Tfh and Treg, each associated with a characteristic transcription factor and cytokine profile [30]. Such transcription factor profiles include T-bet (Th1), GATA-3 (Th2), RORγt (Th17), c-MAF (Tfh) and FoxP3 (Treg). However, a closer look indicates that the expression of a given transcription factor is not always restricted to a particular T-cell subset, that is promiscuous expression patterns are observed [31]. In fact, FoxP3$^+$ Treg cells coexpressing RORγt were found to exert enhanced suppressive activity both *in vitro* and *in vivo* in a model of intestinal inflammation when compared to their RORγt-negative counterparts [32]. Additional studies also pointed to a cytokine environment-dependent role of transcription factors T-bet and GATA-3 in shaping the suppressive function of Treg cells [33]. Not surprisingly, the cytokine and TCR signal strength-dependent differentiation of T cells into functional subsets is regulated by epigenetic mechanisms. An interesting study by Wei *et al.* [34] nicely illustrated the role of histone modifications in T helper subset plasticity. Using high-throughput ChIP-seq, these authors found a set of genes, alternative transcripts and regulatory elements in a genomewide H3K4me3 and H3K27me3 map in murine naïve, Th1, Th2, Th17, naturally occurring Treg (nTreg) and induced Treg (iTreg) cells. Of interest, they found repressive H3K27me3 marks on the *IL-4* gene only in naïve, Th1 and Th17 cells, but not in Treg cells, which accordingly might express the *bona fide* Th2 cytokine IL-4. Similar observations were made for the *Foxp3* locus in Th17 cells. In all T-cell subtypes, most of the signal transducers and activators of transcription (STATs) were marked with H3K4me3 in the promoter region, but without H3K27me3. This, in the case of STAT5, specifically indicated the alternative expression of two isoforms of STAT5b, depending on the fate decision of lineage commitment [34]. Further studies profiled active and poised enhancers in primary human Th1 and Th2 T cells by focusing on the genome-wide distribution of H3K4me2 marks [35]. By comparing Th2 cells from healthy and asthmatic individuals, this approach revealed disease-specific enhancers in Th2 cells which were further linked to combined analysis of single nucleotide polymorphisms (SNPs), transcription factors, microRNAs and gene expression [35]. Alternatively, functional T-cell

© 2017 The Foundation for the Scandinavian Journal of Immunology

commitment can be studied based on the methylation status of signature transcription factor and cytokine loci. As an example, the promoter methylation at the *IL-17A* locus has been used to profile *ex vivo* isolated CD4 T cells in the inflamed joints of patients with rheumatoid arthritis [36]. It should be noted with caution, however, that the transient expression of given transcription factors and associated cytokines cannot clearly identify unambiguous T-cell subset specification. Epigenetic processes have a much more pronounced role in the molecular identification of a given cell. For instance, a well-investigated case is the correlation between DNA methylation status of FoxP3 and the suppressive activity of nTreg cells [37]. As originally reported by J. Huehn and coworkers, there is selective demethylation in an evolutionarily conserved element within the *FoxP3* locus termed Treg-specific demethylated region (TSDR) in naturally occurring Treg (nTreg) but neither in induced Treg (iTreg) nor in activated conventional CD4 T cells (which transiently upregulate FoxP3 protein expression) [38, 39]. Continued demethylation at the TSDR is required to maintain the suppressive function of Treg. Interestingly, however, expression of FoxP3 and induction of epigenetic regulation including hypomethylation are two independent events triggered by TCR stimulation during intrathymic development of nTreg cells [40]. Recent data assign an important role to the genome organizer Satb1 in the epigenetic orchestration of intrathymic Treg development [41]. As reported by Kitagawa and coworkers, Satb1 expression in $CD4^+CD8^+$ double-positive thymocytes or immature $CD4^+$ single-positive thymocytes precedes the induction of Treg precursor cells. Satb1 orchestrates the Treg-specific super-enhancers which then in concert with epigenetic alterations enable the initiation of *FoxP3* expression [41]. Overall, active FoxP3 can act as a transcriptional activator or repressor. A detailed genome-wide DNA methylation analysis has identified a number of hypomethylated genes which are regulated by FoxP3 in human Treg cells. As an example, Zhang *et al.* characterized the T-cell immunoglobulin and immunoreceptor tyrosine-based inhibitory motif domain (TIGIT) as a novel marker of nTreg cells [42].

$\gamma\delta$ T cells versus $\alpha\beta$ T cells: what about epigenetics?

Based on TCR expression, two subpopulations of T cells can be differentiated, that is $\alpha\beta$ and $\gamma\delta$ T cells. $\alpha\beta$ T cells carry a CD3-associated $\alpha\beta$TCR which is generated from a large pool of available Vα and Vβ genes upon somatic TCR gene rearrangement during intrathymic T cell differentiation. In the periphery, most $\alpha\beta$ T cells are either CD4 or CD8 single positive. Mature CD4 and CD8 T cells recognize peptides presented by MHC class II or MHC class I molecules, respectively [43]. A separate lineage of T cells expresses the alternative $\gamma\delta$TCR. The germline-encoded $\gamma\delta$TCR repertoire is much smaller compared to $\alpha\beta$ T cells. Moreover, $\gamma\delta$ T cells recognize different ligands and without MHC restriction. The major population of $\gamma\delta$ T cells in human peripheral blood recognizes microbe- or tumour-derived pyrophosphates ('phosphoantigens') and therefore plays a role in anti-infective and tumour immunity [44]. Like their $\alpha\beta$ T cell counterpart, $\gamma\delta$ T cells display a wide functional plasticity spanning from distinct cytokine expression patterns to regulatory activity and even antigen-presenting capacity [45]. It can be anticipated that epigenetic regulation plays a major role also in controlling $\gamma\delta$ T cell functions (see Fig. 2). Thus, at least two events in intrathymic T cell development are controlled by epigenetic mechanisms, that is the transition from progenitor cells *via* different stages of $CD4^-CD8^-$ double-negative (DN) to $CD4^+CD8^+$ double-positive (DP) thymocytes and eventually mature $CD4^+$ and $CD8^+$ single-positive (SP) T cells, and the acquisition of suitable $\alpha\beta$ or $\gamma\delta$ TCR. While the earliest phase is characterized by high transcriptional activity and gain of permissive H3K4me3 marks, repressive H3K27me3 marks characterize later stages from DN to DP transition, restricting the T-cell specification programme upon progressing differentiation [46]. The zinc finger transcription factor Th-POK was identified as the master regulator of CD4 T cell differentiation. In the DP stage, Th-POK is marked by both H3K4me3 and H3K27me3 and thus is in a bivalent state. This resolves into permissive H3K4me3-only state in CD4 T cells and repressive H3K27me3-only state in CD8 T cells, thus positioning the epigenetic control of *Th-POK* as a central regulator of CD4 versus CD8 T cell branching [47]. This process is further influenced epigenetically by additional transcription factors including Mazr and RUNX family members [48]. Furthermore, the genome organizer Satb1 was recently identified as an essential component in the activation of lineage-specifying transcription factors in the thymus. Satb1 regulates enhancers in these genes in a locus-specific manner and thus is required for appropriate T-cell lineage development [49]. Chromatin remodelling during cellular differentiation is a dynamic process. Comparing wild-type and *Ets1*-deficient thymocytes, Cauchy *et al.* reported that the ability of the transcription factor Ets1 to bind to nucleosomal DNA changes during differentiation, correlating with the expression of a specific transcriptional programme during the DN to DP transition [50]. Importantly, epigenetic regulation during T-cell lineage commitment is not limited to histone modification but similarly involves DNA modifications. DNA methyltransferases (DNMTs) mediate cytosine methylation (5mC), most notably in the CpG islands. In recent years, another DNA modification has been described, mediated by ten-eleven translocation (TET) proteins which catalyse the hydroxylation of 5mC to 5-hydroxymethylcytosine (5hmC) in DNA [51]. A genomewide mapping of 5hmC during sequential steps of thymocyte differentiation was performed by Tsagaratou *et al.* [52]. Based on cytosine-5-

methylensulphonate (CMS) containing DNA immunoprecipitation (CMS-IP) followed by deep sequencing, these authors showed that 5hmC levels in the gene body strongly correlate with histone modifications and gene expression, and change dynamically during T-cell differentiation [52].

Epigenetic regulation is also important for the process of TCR gene rearrangement. For successful V(D)J recombination to occur, the lymphoid-specific RAG1/2 recombinases need to have access to the respective loci, and transcriptional enhancers and promoters control this process *via* the modulation of the chromatin structure [53]. Moreover, it has been shown that high and extended H3K4me3 marks are present throughout the Dβ-Jβ-Cβ gene segments of the *TCRB* locus which depends on RNA polymerase II-mediated transcriptional activity, which together with the extended H3K4 trimethylation renders Dβ and Jβ gene segments highly accessible [54]. It is also important to note that specific *TCRD* gene rearrangements occur in a strict order which is dependent on RUNX proteins. Thus, it was recently shown that RUNX1 mediates the specific targeting of the RAG1 recombinase to the Dδ2-23 recombination signal sequence, and inhibition of RUNX1 binding disrupted the order of TCR-δ rearrangement in suitable *in vitro* differentiation models [55].

Studies on the transcriptional and epigenetic profile of $\gamma\delta$ T cells are limited. Several groups have investigated the transcriptome of human peripheral blood $\gamma\delta$ T cells in comparison with $\alpha\beta$ T cells and NK cells, also with regard to modulation by selected costimuli such as Toll-like receptor ligands [56, 57]. Phosphoantigens such as isopentenyl pyrophosphate (IPP), generated through a dysregulated mevalonate pathway in tumour cells, or microbe-derived phosphoantigens and pathogen-associated molecule patterns from infectious pathogens are sensed by $\gamma\delta$ T cells as a 'danger signals'. Functional studies have also discovered differences in the priming requirements of $\gamma\delta$ T cells as compared to $\alpha\beta$ T cells with regard to induction of selected cytokines. We recently reported that potent IL-9 production is induced in human $\gamma\delta$ T cells by TCR activation in the presence of TGF-β, whereas the induction of IL-9 in $\alpha\beta$ T cells required IL-4 plus TGF-β [58]. Similarly, murine intraepithelial $\gamma\delta$ T cells have been extensively characterized at the transcriptional level [59]. In recent years, $\gamma\delta$ T cells have been also identified as an important source of early IL-17 production. In fact, IL-17- and interferon-γ (IFN-γ)-producing $\gamma\delta$ T cells have been characterized as distinct subsets which develop in the mouse thymus, correlating to some extent with CD27 surface expression. Schmolka *et al.* performed a genome-wide histone (H3) acetylation and methylation profiling of murine IL-17- versus IFN-γ-producing $\gamma\delta$ T cells. These studies showed that both subsets share an early developmental programme but acquire distinct molecular profiles during the functional polarization [60]. Overall, the available data support the notion that CD27$^+$ murine $\gamma\delta$ T cells have a stable IFN-γ-producing phenotype while the CD27$^-$ counterpart displays more functional plasticity [61]. In humans, promiscuous IL-17$^+$/IFN-γ^+ $\gamma\delta$ T cells have been identified under inflammatory conditions [62]. Taken together, it is now widely accepted to classify $\gamma\delta$ T cells as 'innate-like' T cells which can exert an immediate response upon sensing danger signals [44].

We have recently set out to generate a detailed epigenetic landscape of human $\gamma\delta$ T cells in comparison with $\alpha\beta$ T cells. To this end, we compared purified $\gamma\delta$ T cells and $\alpha\beta$ T cell subsets (CD4, CD8, Treg) from healthy individuals at multiple regulatory levels of epigenetic processes. The combined analysis will allow us to identify important genes that are differentially regulated in $\gamma\delta$ and $\alpha\beta$ T cells (Bhat *et al.,* unpublished).

Generation of T-cell memory

Memory is the hallmark of adaptive immunity. During an ongoing immune response, a fraction of T cells differentiate into long-lived memory cells which persist in niches in the bone marrow, and can rapidly respond upon re-encounter of the specific antigen [63]. The differentiation of a T cell from the naïve stage *via* the effector towards the memory stage is accompanied by the expression of sets of cell surface markers and specific transcriptional and epigenetic programmes [64]. Overall, generation of T-cell memory is linked to specific epigenetic events such as DNA methylation and repressive and permissive histone modifications [65]. On a genome-wide level, permissive (H3K4me3) and repressive (H3K27me) histone marks were found to correlate with gene expression in distinct subset of naïve and memory CD8 T cells [66]. Further investigations have characterized the dynamics of permissive and repressive histone marks in *ex vivo* isolated naïve, effector and memory CD8 T cells during an ongoing influenza virus infection. These studies revealed a focused reduction of repressive H3K27me marks in memory CD8 as compared to naïve CD8 T cells [67]. Integrated systems biology approaches based on available GEO data sets have also been used to identify regulatory networks of transcription factors associated with CD8 T cell memory development. In addition to known transcription factors, these studies have helped to identify novel key factors for this process such as *Bach2* [68]. A recent study by Yu *et al.* applying a multilayer epigenetic approach including RNA-seq, ChIP-seq and ATAC-seq to study gene expression, genome-wide histone modifications and chromatin accessibility additionally identified transcription factors with novel roles in CD8 T cell differentiation. Specifically, these authors found that the nuclear glucocorticoid receptor nuclear receptor subfamily 3 group C member 1 (Nr3c1) regulates memory precursor CD8 T cells during infection with the intracellular microbe *Listeria monocytogenes* [18]. In addition to histone modifications, acquired changes in DNA

© 2017 The Foundation for the Scandinavian Journal of Immunology

methylation characterize memory CD8 T cells. Using whole-genome sequencing of bisulphite-converted DNA, Abdelsamed and coworkers recently reported that human memory CD8 T cells maintain their subset-specific DNA methylation pattern upon cytokine-driven, antigen-independent *in vitro* proliferation and also upon adoptive transfer into transplant patients [69].

Similar to CD8 T cells, epigenetic regulation also controls memory development in the CD4 T cell compartment. Kinkley and coworkers developed a modified ChIP-seq method which helped to identify bivalent HeK4me3 and H3K27me3 marks in human CD4 memory T cells [70]. A deep epigenomic profiling of human CD4 T cell subsets addressing DNA methylation, histone modifications, chromatin accessibility and coding and regulatory RNAs has defined specific characteristics of naïve (T_N), central memory (T_{CM}), effector memory (T_{EM}) and terminally differentiated CD45RA$^+$ (T_{EMRA}) CD4 T cells [71]. These studies have not only defined critical factors controlling checkpoints for naïve CD4 T cells (such as FOXP1) but also suggest a linear differentiation model of CD4 T cells during an ongoing immune response ($T_N \rightarrow T_{CM} \rightarrow T_{EM} \rightarrow T_{EMRA}$) based on transcriptome analysis, chromatin accessibility and DNA methylation [71]. Importantly, epigenetic mechanisms also regulate the expression of homing receptors on CD4 memory T cells. As shown by Szilagyi *et al.,* expression of the mucosa-specific homing receptor $\alpha_4\beta_7$ integrin on murine CD4 memory T cells is maintained also in the absence of inducing factors, due to selective DNA demethylation [72].

As briefly discussed here, there is no doubt that epigenetic mechanisms are essential for memory T-cell responses and development. Despite enormous progress, however, many questions remain open at this point. As an example, the role of 'super-enhancers' in the maintenance of memory T cell function after cell division remains to be specified in more detail [73].

Epigenetics of T cells in autoimmunity

Autoimmune diseases are characterized by inappropriate immune regulation, thus allowing autoreactive B and/or T cells to develop into effector cells. Not surprisingly, T cell function in autoimmune diseases like rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) or multiple sclerosis (MS) is also regulated by epigenetic mechanisms [74]. Given the exceptional role of Treg cells in controlling peripheral tolerance, altered epigenetic modification affecting *FoxP3* (e.g. demethylation at the TSDR region) or other genes controlling Treg activity may contribute to the pathogenesis of autoimmune diseases [75] (see Fig. 3). MS is usually considered to be a CD4 T cell-mediated (or at least dependent) autoimmune disease. Sanders *et al.* recently compared miRNA profiles of CD4 T cells from patients with secondary progressive MS with healthy donors. Results obtained by NGS and validated by RT-qPCR identified several miRNAs specifically downregulated in MS CD4 T cells, with the suppressor of cytokine signalling 6 (SOCS6) being one gene targeted by eight of ten of the identified miRNAs [76]. Moreover, genomewide DNA methylation studies have identified DMRs of CD4 T cells in MS patients compared to healthy controls, specifically on chromosome 6p21 at HLA-DRB1 (a known MS risk locus), and additional non-HLA CpGs that localized to

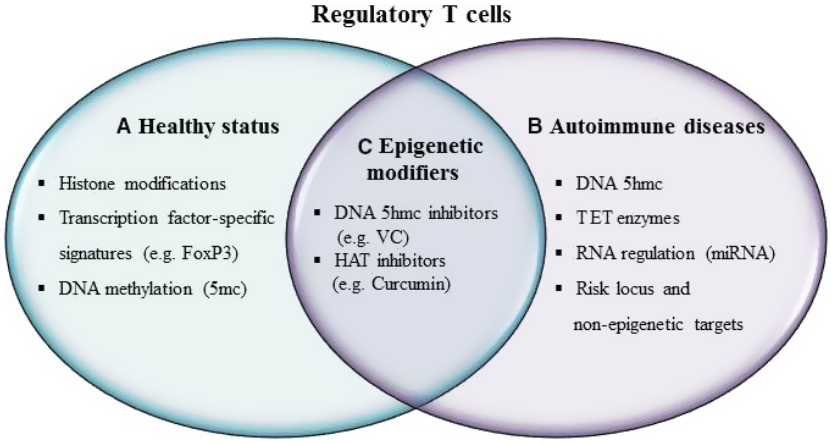

Figure 3 The importance of epigenetic mechanisms for regulatory T cells. (A) Histone modifications, DNA methylation and transcriptional regulation have been well documented to determine Treg differentiation in the healthy state. (B) Furthermore, studies focused on autoimmune diseases such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) or multiple sclerosis (MS) explore the importance of regulation of epigenetic (e.g. TET enzymes) and non-epigenetic proteins (e.g. CD300LB). Additionally, addressing risk loci and regulatory RNA (e.g. miRNA) may help to understand the pathophysiology of diseases. (C) On the intersection of fundamental epigenetic processes explored in the healthy versus diseased state, promising targets for therapeutic intervention can be discovered. Treg, regulatory T cells; 5mc, DNA 5-methylcytosine; VC, vitamin C; HAT, histone acetyltransferase; TET, ten-eleven translocation.

genes linked with MS [77]. Interestingly, the methylation profile of CD8 T cells in the same cohort of patients with relapsing remitting MS differed from CD4 T cells, supporting the notion that it is important to consider cell type-specific epigenetic regulation [78]. Mouse models of MS illustrate how targeting epigenetic mechanisms can also contribute to the design of novel therapies. The hypomethylating agent decitabine was found to ameliorate clinical symptoms in experimental allergic encephalomyelitis (EAE), associated with increased production of anti-inflammatory cytokines and induction of Treg cells [79]. On the other hand, DNA demethylation can also promote autoimmunity as shown in lupus-prone MRL/lpr mice by Wu and coworkers. These authors observed activation of follicular helper T cells (Tfh) together with enhanced lupus symptoms in these mice upon high sodium chloride diet. They found that Tfh polarization was due to DNA demethylation as a consequence of NaCl-induced recruitment of TET enzymes [80]. Widespread epigenetic alterations are also present in CD4 T cells from SLE patients compared to age- and sex-matched healthy controls. Thus, specific changes in DNA methylation correlating with clinical phenotypes were detected in novel key target genes including *NLRP2*, *CD300LB* and *S1PR3* [81]. Moreover, CD4 T cells from SLE patients display upregulated expression of TET2/3 enzymes, and increased 5hmC levels were found in the promoter regions of many immune-related genes, in agreement with upregulated gene expression [82]. It appears that disease-associated changes in the genome-wide DNA methylation pattern are present as well in other autoimmune diseases including RA [83, 84], Grave's disease [85] or Behçet's disease [86]. In addition to T cells, such alterations can also extend to B cells [83, 84] or monocytes [86]. Overall, these few examples illustrate that epigenetic mechanisms are involved in many if not most autoimmune diseases but also offer novel targets for therapeutic intervention [87]. However, it should be kept in mind that the precise role of epigenetic processes in the pathogenesis is not known for most autoimmune diseases, in part due to relatively small patient cohorts.

Epigenetic drugs

The epigenetic code is executed by enzymes targeting histone modifications (such as histone deacetylases, HDAC; or histone acetyl transferases, HAT) and DNA methylation (such as DNA methyltransferases, DNMT; and TET enzymes). Corresponding inhibitors and activators interfere with epigenetic regulation and may have profound effects on T-cell differentiation and immune responses (see Figs 2 and 3). Demethylation at the TSDR region of *FoxP3* is required for sustained Treg activity. In line, the DNMT inhibitor decitabine (5-aza-2′-deoxycytidine) was found to induce and stabilize FoxP3 expression and Treg activity in murine and human CD4 T cells [88, 89]. Interestingly, similar effects can be mediated by vitamin C (VC). VC does not only have strong antioxidant activity but also increases TET enzyme activity and thereby DNA hydroxymethylation. This results in FoxP3 demethylation and stabilization and thereby preserved Treg activity [90, 91]. Our own studies indicate that VC can enhance and stabilize the expression of FoxP3 and selected other transcription factors also in human peripheral blood $\gamma\delta$ T cells (Kouakanou *et al.*, unpublished). In addition, it was recently observed that VC can also induce histone modifications. As reported by Song *et al.,* VC enhanced the expression of IL-17 in murine CD4 T cells by modulating the histone deacetylase Jumonji C domain-containing protein 2 (Jmjd2), thereby reducing H3K9me3 marks in the *IL-17* gene [92].

Apart from DNMT inhibitors, several other substances are investigated in T-cell epigenetics. These include epigallocatechin gallate (EGCG), a polyphenol present in green tea [93, 94], TET activator vitamin C, the natural product curcumin, a CBP/p300-specific HAT inhibitor, and the chemically synthesized HAT inhibitor CBP30. Curcumin and CBP are reported to induce anti-inflammatory response by inhibiting NFκB and AP-1 transcription factors. Curcumin inhibited release of chemokines like MIP-1α, MIP-1β and RANTES in human $\gamma\delta$ T cells [95], while CBP30 inhibited IL-17A secretion by Th17 cells from healthy human individuals and patients with ankylosing spondylitis and psoriatic arthritis [96].

The HDAC inhibitor valproic acid (VPA) is in clinical use for the treatment of epilepsy and bipolar disorders but is also widely used to interfere with epigenetic regulation. When comparing human peripheral blood $\alpha\beta$ and $\gamma\delta$ T cells, we observed substantial differences in the modulation of some surface markers by VPA. Cell surface antigens which were substantially more affected in $\gamma\delta$ T cells included CD86, CD54 and NKG2D [97]. We also found that VPA induced the expression of a non-secreted isoform of IL-4 (IL-4$_\delta$13) in $\gamma\delta$ T cells which was linked to histone H3 acetylation and regulation of the c-Jun transcription factor network [98]. VPA as well as newly emerging other HDAC inhibitors and DNMT inhibitors have multiple direct effects on tumour cells, but accumulating evidence indicates that they may additionally modulate the antitumour immune response, for instance *via* the NKG2D receptor/ligand interaction. The activating NKG2D receptor is expressed on CD8 T cells, NK cells and $\gamma\delta$ T cells but rarely on CD4 T cells. In line, *NKG2D* is hypermethylated (i.e. silenced) in CD4 T cells whereas it is hypomethylated in CD8 T cells, which also correlates with the H3K9ac status [99]. NKG2D ligands including MICA/B and ULBP family members are not present on normal cells but are constitutively expressed on some but not all transformed cells where they serve as targets for recognition by NKG2D-expressing cytotoxic effector cells. *MIC* genes might be silenced through promoter hypoacetylation, as shown for Merkel cell carcinoma (MCC), in which

© 2017 The Foundation for the Scandinavian Journal of Immunology

case MICA/B expression and thus susceptibility to NKG2D-dependent cytotoxicity could be reinstalled by HDAC inhibitors like Vorinostat and Mithramycin A [100]. Not unexpectedly, HDAC inhibitors also enhance the expression of other cell surface (e.g. HLA class I and ICAM-1) and intracellular molecules (e.g. LMP2, calnexin, β2-microglobulin) in tumour cells important for recognition by T cells and thereby can enhance T-cell-mediated lysis of tumour cells [101]. In view of the diverse effects of HDAC inhibitors and other epigenetic modifiers, it is tempting to explore possible synergies with other well-established antitumour therapies. Along this line, it was recently shown that the HDAC inhibitor panobinostat enhances the tumoricidal effect of anti-HER2 antibody trastuzumab through direct effects on tumour cells but also *via* enhancing antibody-dependent cytotoxicity [102]. Moreover, checkpoint inhibitors such as anti-PD-1 antibody in combination with HDAC inhibitors are under intensive investigation in various preclinical studies and clinical trials [103]. As an example, several clinical studies are underway with anti-PD-1 antibodies pembrolizumab or nivolumab, or anti-PDL-1 antibody atezolizumab in combination with HDAC inhibitor entinostat and/or DNMT inhibitor azacytidine (NCT02437136; NCT02697630; NCT01928576; NCT02453620; NCT02708680; NCT01928576; http://clinicaltrials.gov). In a mouse lung cancer model, Zheng *et al.* showed that the combination of romidepsin (a selective class I HDAC inhibitor) and anti-PD-1 treatment promotes a positive chemokine–IFN-γ feedback loop through tumour cells and T-cell intermediaries [104]. Notably, the synergistic effect of both romidepsin and PD-1 blockade leads to enhanced T-cell infiltration of tumours and increased T-cell functionality. While HDAC inhibitors might thus have perspectives for the treatment of certain cancer types, more in-depth studies are required. Our own work showing enhanced shedding of NKG2D ligands from tumour cells in response to HDAC inhibitors (Bhat *et al.,* submitted for publication) and other studies pointing to a dual role of HDAC in tumorigenesis according to the stage of tumour progression [105] raise a note of caution.

Future perspectives

As we have discussed here, epigenetic regulation is an essential component of T cell differentiation [106]. Technological progress has opened new possibilities to study epigenetic regulation even at the single-cell level, thereby paving the way for deeper insights into normal and pathological T cell differentiation. Thus, future studies will allow detailed investigations of single T cells at different localizations, for instance in inflamed tissues or in the tumour microenvironment. Another important development is the potential use of epigenetic modifiers to enhance immune responses *in vivo* [107]. Detailed studies are warranted to find out under which conditions HDAC inhibitors used in the clinic can enhance antitumour immune responses, also in the context of tumour vaccination. Similarly, modulators of DNA methylation and hydroxymethylation might offer interesting perspectives for clinical application. As an example, it has been demonstrated that decitabine can upregulate the expression of NKG2D ligands [108]. In addition, decitabine may support antitumour immune responses by mechanisms unrelated to epigenetic regulation, for example by depleting myeloid-derived suppressor cells [109]. Vitamin C has been shown to stabilize FoxP3 expression in Treg cells *via* TET proteins and thus may prove useful to enhance Treg activity in certain conditions such as autoimmune diseases. These few examples illustrate that we currently witness an exciting period where results of basic research in T-cell epigenetics help to shape novel therapeutic strategies.

Acknowledgment

Work from the author's laboratories was supported by the Cluster of Excellence Inflammation at Interfaces (EXC 306) funded by the German Research Foundation (DK, OA), by DFG Ka 502/19-1 (DK) and by Else-Kröner Fresenius Foundation 2013_A276 (DK). JB, GC and LK were supported by grants from the Werner and Klara Kreitz Foundation and CP and GC by grants from Kiel University Medical Faculty. LK acknowledges the receipt of a long-term fellowship from the German Academic Exchange Service (DAAD).

References

1 Ramachandran S, Henikoff S. Nucleosome dynamics during chromatin remodeling in vivo. *Nucleus* 2016;7:20–6.
2 Plongthongkum N, Diep DH, Zhang K. Advances in the profiling of DNA modifications: cytosine methylation and beyond. *Nat Rev Genet* 2014;15:647–61.
3 Iurlaro M, Ficz G, Oxley D *et al.* A screen for hydroxymethylcytosine and formylcytosine binding proteins suggests functions in transcription and chromatin regulation. *Genome Biol* 2013;14:R119.
4 Sampath SC, Marazzi I, Yap KL *et al.* Methylation of a histone mimic within the histone methyltransferase G9a regulates protein complex assembly. *Mol Cell* 2007;27:596–608.
5 Nicodeme E, Jeffrey KL, Schaefer U *et al.* Suppression of inflammation by a synthetic histone mimic. *Nature* 2010;468:1119–23.
6 Quinn JJ, Chang HY. Unique features of long non-coding RNA biogenesis and function. *Nat Rev Genet* 2016;17:47–62.
7 Roundtree IA, Evans ME, Pan T, He C. Dynamic RNA modifications in gene expression regulation. *Cell* 2017;169:1187–200.
8 Prabakaran S, Lippens G, Steen H, Gunawardena J. Post-translational modification: nature's escape from genetic imprisonment and the basis for dynamic information encoding. *Wiley Interdiscip Rev Syst Biol Med* 2012;4:565–83.
9 Tang F, Barbacioru C, Nordman E *et al.* RNA-Seq analysis to capture the transcriptome landscape of a single cell. *Nat Protoc* 2010;5:516–35.
10 Aune TM, Crooke PS 3rd, Spurlock CF 3rd. Long noncoding RNAs in T lymphocytes. *J Leukoc Biol* 2016;99:31–44.

11 Amado T, Schmolka N, Metwally H, Silva-Santos B, Gomes AQ. Cross-regulation between cytokine and microRNA pathways in T cells. *Eur J Immunol* 2015;45:1584–95.

12 Mundade R, Ozer HG, Wei H, Prabhu L, Lu T. Role of ChIP-seq in the discovery of transcription factor binding sites, differential gene regulation mechanism, epigenetic marks and beyond. *Cell Cycle* 2014;13:2847–52.

13 Roh TY, Cuddapah S, Cui K, Zhao K. The genomic landscape of histone modifications in human T cells. *Proc Natl Acad Sci USA* 2006;103:15782–7.

14 Buenrostro JD, Giresi PG, Zaba LC, Chang HY, Greenleaf WJ. Transposition of native chromatin for fast and sensitive epigenomic profiling of open chromatin, DNA-binding proteins and nucleosome position. *Nat Methods* 2013;10:1213–18.

15 Buenrostro JD, Wu B, Litzenburger UM *et al.* Single-cell chromatin accessibility reveals principles of regulatory variation. *Nature* 2015;523:486–90.

16 Chen X, Shen Y, Draper W *et al.* ATAC-see reveals the accessible genome by transposase-mediated imaging and sequencing. *Nat Methods* 2016;13:1013–120.

17 Kartashov AV, Barski A. BioWardrobe: an integrated platform for analysis of epigenomics and transcriptomics data. *Genome Biol* 2015;16:158.

18 Yu B, Zhang K, Milner JJ *et al.* Epigenetic landscapes reveal transcription factors that regulate $CD8^+$ T cell differentiation. *Nat Immunol* 2017;18:573–82.

19 Hodges E, Molaro A, Dos Santos CO *et al.* Directional DNA methylation changes and complex intermediate states accompany lineage specificity in the adult hematopoietic compartment. *Mol Cell* 2011;44:17–28.

20 Lara-Astiaso D, Weiner A, Lorenzo-Vivas E *et al.* Chromatin state dynamics during blood formation. *Science* 2014;345:943–9.

21 Zilbauer M, Rayner TF, Clark C *et al.* Genome-wide methylation analyses of primary human leukocyte subsets identifies functionally important cell-type-specific hypomethylated regions. *Blood* 2013;122:e52–60.

22 Chen L, Ge B, Casale FP *et al.* Genetic drivers of epigenetic and transcriptional variation in human immune cells. *Cell* 2016;167:1398–414.

23 Ecker S, Chen L, Pancaldi V *et al.* Genome-wide analysis of differential transcriptional and epigenetic variability across human immune cell types. *Genome Biol* 2017;18:18.

24 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;9:429–45.

25 Zhao M, Qin J, Yin H *et al.* Distinct epigenomes in $CD4^+$ T cells of newborns, middle-ages and centenarians. *Sci Rep* 2016;6:38411.

26 Tu W, Rao S. Mechanisms underlying T cell immunosenescence: aging and cytomegalovirus infection. *Front Microbiol* 2016;7:2111.

27 Suarez-Álvarez B, Rodríguez RM, Schlangen K *et al.* Phenotypic characteristics of aged $CD4^+$ $CD28^{null}$ T lymphocytes are determined by changes in the whole-genome DNA methylation pattern. *Aging Cell* 2017;16:293–303.

28 Moskowitz DM, Zhang DW, Hu B *et al.* Epigenomics of human CD8 T cell differentiation and aging. *Sci Immunol.* 2017;2: pii: eaag0192. https://doi.org/10.1126/sciimmunol.aag0192. Epub 2017 Feb 1

29 Galindo-Albarrán AO, López-Portales OH, Gutiérrez-Reyna DY *et al.* $CD8^+$ T cells from human neonates are biased toward an innate immune response. *Cell Rep* 2016;17:2151–60.

30 Caza T, Landas S. Functional and phenotypic plasticity of $CD4^+$ T cell subsets. *Biomed Res Int* 2015;2015:521957.

31 Billerbeck E, Labitt RN, Vega K *et al.* Insufficient interleukin-12 signalling favours differentiation of human $CD4^+$ and $CD8^+$ T cells into GATA-3^+ and GATA-3^+ T-bet^+ subsets in humanized mice. *Immunology* 2014;143:202–18.

32 Yang BH, Hagemann S, Mamareli P *et al.* Foxp3^+ T cells expressing RORγt represent a stable regulatory T-cell effector lineage with enhanced suppressive capacity during intestinal inflammation. *Mucosal Immunol* 2016;9:444–57.

33 Yu F, Sharma S, Edwards J, Feigenbaum L, Zhu J. Dynamic expression of transcription factors T-bet and GATA-3 by regulatory T cells maintains immunotolerance. *Nat Immunol* 2015;16:197–206.

34 Wei G, Wei L, Zhu J *et al.* Global mapping of H3K4me3 and H3K27me3 reveals specificity and plasticity in lineage fate determination of differentiating $CD4^+$ T cells. *Immunity* 2009;30:155–67.

35 Seumois G, Chavez L, Gerasimova A *et al.* Epigenomic analysis of primary human T cells reveals enhancers associated with TH2 memory cell differentiation and asthma susceptibility. *Nat Immunol* 2014;15:777–88.

36 Janson PC, Linton LB, Bergman EA *et al.* Profiling of $CD4^+$ T cells with epigenetic immune lineage analysis. *J Immunol* 2011;186:92–102.

37 Huehn J, Beyer M. Epigenetic and transcriptional control of Foxp3^+ regulatory T cells. *Semin Immunol* 2015;27:10–18.

38 Baron U, Floess S, Wieczorek G *et al.* DNA demethylation in the human FOXP3 locus discriminates regulatory T cells from activated FOXP3^+ conventional T cells. *Eur J Immunol* 2007;37:2378–89.

39 Polansky JK, Kretschmer K, Freyer J *et al.* DNA methylation controls Foxp3 gene expression. *Eur J Immunol* 2008;38:1654–63.

40 Ohkura N, Hamaguchi M, Morikawa H *et al.* T cell receptor stimulation-induced epigenetic changes and Foxp3 expression are independent and complementary events required for Treg cell development. *Immunity* 2012;37:785–99.

41 Kitagawa Y, Ohkura N, Kidani Y *et al.* Guidance of regulatory T cell development by Satb1-dependent super-enhancer establishment. *Nat Immunol* 2017;18:173–83.

42 Zhang Y, Maksimovic J, Naselli G *et al.* Genome-wide DNA methylation analysis identifies hypomethylated genes regulated by FOXP3 in human regulatory T cells. *Blood* 2013;122:2823–36.

43 Clambey ET, Davenport B, Kappler JW, Marrack P, Homann D. Molecules in medicine mini review: the αβ T cell receptor. *J Mol Med (Berl)* 2014;92:735–41.

44 Vantourout P, Hayday A. Six-of-the-best: unique contributions of gammadelta T cells to immunology. *Nat Rev Immunol* 2013;13:88–100.

45 Kabelitz D, He W. The multifunctionality of human Vγ9Vδ2 γδ T cells: clonal plasticity or distinct subsets? *Scand J Immunol* 2012;76:213–22.

46 Naito T, Taniuchi I. Roles of repressive epigenetic machinery in lineage decision of T cells. *Immunology* 2013;139:151–7.

47 Vigano MA, Ivanek R, Balwierz P *et al.* An epigenetic profile of early T-cell development from multipotent progenitors to committed T-cell descendants. *Eur J Immunol* 2014;44:1181–93.

48 Issuree PD, Ng CP, Littman DR. Heritable gene regulation in the CD4:CD8 T cell lineage choice. *Front Immunol* 2017;8:291.

49 Kakugawa K, Kojo S, Tanaka H *et al.* Essential roles of SATB1 in specifying T lymphocyte subsets. *Cell Rep* 2017;19:1176–88.

50 Cauchy P, Maqbool MA, Zacarias-Cabeza J *et al.* Dynamic recruitment of Ets1 to both nucleosome-occupied and -depleted enhancer regions mediates a transcriptional program switch during early T-cell differentiation. *Nucleic Acids Res* 2016;44:3567–85.

51 Pastor WA, Aravind L, Rao A. TETonic shift: biological roles of TET proteins in DNA demethylation and transcription. *Nat Rev Mol Cell Biol* 2013;14:341–56.

52 Tsagaratou A, Äijö T, Lio CW *et al.* Dissecting the dynamic changes of 5-hydroxymethylcytosine in T-cell development and differentiation. *Proc Natl Acad Sci U S A* 2014;111:e3306–15.

© 2017 The Foundation for the Scandinavian Journal of Immunology

53 Spicuglia S, Zacarias-Cabeza J, Pekowska A, Ferrier P. Epigenetic regulation of antigen receptor gene rearrangement. *F1000 Biol Rep* 2010;2:23. https://doi.org/10.3410/b2-23

54 Zacarías-Cabeza J, Belhocine M, Vanhille L *et al.* Transcription-dependent generation of a specialized chromatin structure at the TCRβ locus. *J Immunol* 2015;194:3432–43.

55 Cieslak A, Le Noir S, Trinquand A *et al.* RUNX1-dependent RAG1 deposition instigates human TCR-δ locus rearrangement. *J Exp Med* 2014;211:1821–32.

56 Pont F, Familiades J, Dejean S *et al.* The gene expression profile of phosphoantigen-specific human $\gamma\delta$ T lymphocytes is a blend of $\alpha\beta$ T-cell and NK-cell signatures. *Eur J Immunol* 2012;42:228–40.

57 Pietschmann K, Beetz S, Welte S *et al.* Toll-like receptor expression and function in subsets of human $\gamma\delta$ T lymphocytes. *Scand J Immunol* 2009;70:245–55.

58 Peters C, Häsler R, Wesch D, Kabelitz D. Human Vδ2 T cells are a major source of interleukin-9. *Proc Natl Acad Sci U S A* 2016;113:12520–5.

59 Fahrer AM, Konigshofer Y, Kerr EM *et al.* Attributes of $\gamma\delta$ intraepithelial lymphocytes as suggested by their transcriptional profile. *Proc Natl Acad Sci USA* 2001;98:10261–6.

60 Schmolka N, Serre K, Grosso AR *et al.* Epigenetic and transcriptional signatures of stable versus plastic differentiation of proinflammatory gammadelta T cell subsets. *Nat Immunol* 2013;14:1093–10.

61 Schmolka N, Wencker M, Hayday AC, Silva-Santos B. Epigenetic and transcriptional regulation of $\gamma\delta$ T cell differentiation: Programming cells for responses in time and space. *Semin Immunol* 2015;27:19–25.

62 Ness-Schwickerath KJ, Morita CT. Regulation and function of IL-17A- and IL-22-producing $\gamma\delta$ T cells. *Cell Mol Life Sci* 2011;68:2371–90.

63 Di Rosa F. Two niches in the bone marrow: a hypothesis on life-long T cell memory. *Trends Immunol* 2016;37:503–12.

64 Youngblood B, Hale JS, Ahmed R. T-cell memory differentiation: insights from transcriptional signatures and epigenetics. *Immunology* 2013;139:277–84.

65 Dogra P, Ghoneim HE, Abdelsamed HA, Youngblood B. Generating long-lived CD8$^+$ T-cell memory: Insights from epigenetic programs. *Eur J Immunol* 2016;46:1548–62.

66 Araki Y, Wang Z, Zang C *et al.* Genome-wide analysis of histone methylation reveals chromatin state-based regulation of gene transcription and function of memory CD8$^+$ T cells. *Immunity* 2009;30:912–25.

67 Russ BE, Olshanksy M, Smallwood HS *et al.* Distinct epigenetic signatures delineate transcriptional programs during virus-specific CD8$^+$ T cell differentiation. *Immunity* 2014;41:853–65.

68 Hu G, Chen J. A genome-wide regulatory network identifies key transcription factors for memory CD8$^+$ T-cell development. *Nat Commun* 2013;4:2830.

69 Abdelsamed HA, Moustaki A, Fan Y *et al.* Human memory CD8 T cell effector potential is epigenetically preserved during in vivo homeostasis. *J Exp Med* 2017;214:1593–606.

70 Kinkley S, Helmuth J, Polansky JK *et al.* re-ChIP-seq reveals widespread bivalency of H3K4me3 and H3K27me3 in CD4$^+$ memory T cells. *Nat Commun* 2016;7:12514.

71 Durek P, Nordström K, Gasparoni G *et al.* Epigenomic profiling of human CD4$^+$ T cells supports a linear differentiation model and highlights molecular regulators of memory development. *Immunity* 2016;45:1148–61.

72 Szilagyi BA, Triebus J, Kressler C *et al.* Gut memories do not fade: epigenetic regulation of lasting gut homing receptor expression in CD4$^+$ memory T cells. *Mucosal Immunol* 2017; https://doi.org/10.1038/mi.2017.7. [Epub ahead of print]

73 Bevington SL, Cauchy P, Cockerill PN. Chromatin priming elements establish immunological memory in T cells without activating transcription: T cell memory is maintained by DNA elements which stably prime inducible genes without activating steady state transcription. *BioEssays* 2017;39:16001884. https://doi.org/10.1002/bies.201600184.

74 Wang Z, Chang C, Lu Q. Epigenetics of CD4$^+$ T cells in autoimmune diseases. *Curr Opin Rheumatol* 2017;29:361–8.

75 Shu Y, Hu Q, Long H, Chang C, Lu Q, Xiao R. Epigenetic variability of CD4$^+$CD25$^+$ Tregs contributes to the pathogenesis of autoimmune diseases. *Clin Rev Allergy Immunol* 2017;52:260–72.

76 Sanders KA, Benton MC, Lea RA *et al.* Next-generation sequencing reveals broad down-regulation of microRNAs in secondary progressive multiple sclerosis CD4$^+$ T cells. *Clin Epigenetics* 2016;8:87.

77 Graves MC, Benton M, Lea RA *et al.* Methylation differences at the HLA-DRB1 locus in CD4$^+$ T-Cells are associated with multiple sclerosis. *Mult Scler* 2014;20:1033–41.

78 Maltby VE, Graves MC, Lea RA *et al.* Genome-wide DNA methylation profiling of CD8$^+$ T cells shows a distinct epigenetic signature to CD4$^+$ T cells in multiple sclerosis patients. *Clin Epigenetics* 2015;7:118.

79 Mangano K, Fagone P, Bendtzen K *et al.* Hypomethylating agent 5-aza-2'-deoxycytidine (DAC) ameliorates multiple sclerosis in mouse models. *J Cell Physiol* 2014;229:1918–25.

80 Wu H, Huang X, Qiu H *et al.* High salt promotes autoimmunity by TET2-induced DNA demethylation and driving the differentiation of Tfh cells. *Sci Rep* 2016;6:28065.

81 Zhao M, Liu S, Luo S *et al.* DNA methylation and mRNA and microRNA expression of SLE CD4$^+$ T cells correlate with disease phenotype. *J Autoimmun* 2014;54:127–36.

82 Zhao M, Wang J, Liao W *et al.* Increased 5-hydroxymethylcytosine in CD4$^+$ T cells in systemic lupus erythematosus. *J Autoimmun* 2016;69:64–73.

83 Glossop JR, Emes RD, Nixon NB *et al.* Genome-wide DNA methylation profiling in rheumatoid arthritis identifies disease-associated methylation changes that are distinct to individual T- and B-lymphocyte populations. *Epigenetics* 2014;9:1228–37.

84 Glossop JR, Emes RD, Nixon NB *et al.* Genome-wide profiling in treatment-naive early rheumatoid arthritis reveals DNA methylome changes in T and B lymphocytes. *Epigenomics* 2016;8:209–24.

85 Limbach M, Saare M, Tserel L *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.

86 Hughes T, Ture-Ozdemir F, Alibaz-Oner F, Coit P, Direskeneli H, Sawalha AH. Epigenome-wide scan identifies a treatment-responsive pattern of altered DNA methylation among cytoskeletal remodeling genes in monocytes and CD4$^+$ T cells from patients with Behçet's disease. *Arthritis Rheumatol* 2014;66:1648–58.

87 Gray SG. Epigenetic-based immune intervention for rheumatic diseases. *Epigenomics* 2014;6:253–71.

88 Lal G, Zhang N, van der Touw W *et al.* Epigenetic regulation of Foxp3 expression in regulatory T cells by DNA methylation. *J Immunol* 2009;182:259–73.

89 Hu Y, Cui Q, Gu Y *et al.* Decitabine facilitates the generation and immunosuppressive function of regulatory $\gamma\delta$T cells derived from human peripheral blood mononuclear cells. *Leukemia* 2013;27:1580–5.

90 Nair VS, Song MH, Oh KI. Vitamin C facilitates demethylation of the Foxp3 enhancer in a Tet-dependent manner. *J Immunol* 2016;196:2119–31.

91 Yue X, Trifari S, Aijo T *et al.* Control of Foxp3 stability through modulation of TET activity. *J Exp Med* 2016;213:377–97.

92 Song MH, Nair VS, Oh KI. Vitamin C enhances the expression of IL17 in an Jmjd2-dependent manner. *BMB Rep* 2017;50:49–54.

93 Wong CP, Nguyen LP, Noh SK, Bray TM, Bruno RS, Ho E. Induction of regulatory T cells by green tea polyphenol EGCG. *Immunol Letters* 2011;139:7–13.

94 Kehrmann J, Tatura R, Zeschnigk M *et al.* Impact of 5-aza-2'-deoxycytidine and epigallocatechin-3-gallate for induction of human regulatory T cells. *Immunology* 2014;142:384–95.
95 Cipriani B, Borsellino G, Knowles H *et al.* Curcumin inhibits activation of Vγ9Vδ2 T cells by phosphoantigens and induces apoptosis involving apoptosis-inducing factor and large scale DNA fragmentation. *J Immunol* 2001;167:3454–62.
96 Hammitzsch A, Tallant C, Fedorov O *et al.* CBP30, a selective CBP/p300 bromodomain inhibitor, suppresses human Th17 responses. *Proc Natl Acad Sci U S A.* 2015;112:10768–73.
97 Bhat J, Oberg HH, Kabelitz D. Modulation of human γδ T-cell activation and phenotype by histone deacetylase inhibitors. *Cell Immunol* 2015;296:50–6.
98 Bhat J, Sosna J, Fritsch J *et al.* Expression of non-secreted IL-4 is associated with HDAC inhibitor-induced cell death, histone acetylation and c-Jun regulation in human γδ T-cells. *Oncotarget* 2016;7:64743–56.
99 Fernandez-Sanchez A, Baragano Raneros A, Carvajal Palao R *et al.* DNA demethylation and histone H3K9 acetylation determine the active transcription of the NKG2D gene in human $CD8^+$ T and NK cells. *Epigenetics* 2013;8:66–78.
100 Ritter C, Fan K, Paulson KG, Nghiem P, Schrama D, Becker JC. Reversal of epigenetic silencing of MHC class I chain-related protein A and B improves immune recognition of Merkel cell carcinoma. *Sci Rep* 2016;6:21678.
101 Gameiro SR, Malamas AS, Tsang KY, Ferrone S, Hodge JW. Inhibitors of histone deacetylase 1 reverse the immune evasion phenotype to enhance T-cell mediated lysis of prostate and breast carcinoma cells. *Oncotarget* 2016;7:7390–402.
102 Medon M, Vidacs E, Vervoort SJ *et al.* HDAC inhibitor panobinostat engages host innate immune defenses to promote the tumoricidal effects of trastuzumab in $HER2^+$ tumors. *Cancer Res* 2017;77:2594–606.
103 Beg AA, Gray JE. HDAC inhibitors with PD-1 blockade: a promising strategy for treatment of multiple cancer types? *Epigenomics* 2016;8:1015–17.
104 Zheng H, Zhao W, Yan C *et al.* HDAC inhibitors enhance T-cell chemokine expression and augment response to PD-1 immunotherapy in lung adenocarcinoma. *Clin Cancer Res* 2016;22:4119–32.
105 Santoro F, Botrugno OA, Dal Zuffo R *et al.* A dual role for Hdac1: oncosuppressor in tumorigenesis, oncogene in tumor maintenance. *Blood* 2013;121:3459–68.
106 Leoni C, Vincenzetti L, Emming S, Monticelli S. Epigenetics of T lymphocytes in health and disease. *Swiss Med Wkly* 2015;145:w14191.
107 Bhat J, Kabelitz D. γδ T cells and epigenetic drugs: A useful merger in cancer immunotherapy? *Oncoimmunology* 2015;4:e1006088.
108 Tang KF, He CX, Zeng GL *et al.* Induction of MHC class I-related chain B (MICB) by 5-aza-2'-deoxycytidine. *Biochem Biophys Res Commun* 2008;370:578–83.
109 Zhou J, Yao Y, Shen Q, Li G, Hu L, Zhang X. Demethylating agent decitabine disrupts tumor-induced immune tolerance by depleting myeloid-derived suppressor cells. *J Cancer Res Clin Oncol* 2017;143:1371–80.
110 Sen DR, Kaminski J, Barnitz RA *et al.* The epigenetic landscape of T cell exhaustion. *Science* 2016;354:1165–9.
111 Schones DE, Cui K, Cuddapah S *et al.* Dynamic regulation of nucleosome positioning in the human genome. *Cell* 2008;132:887–98.
112 Boyle AP, Davis S, Shulha HP *et al.* High-resolution mapping and characterization of open chromatin across the genome. *Cell* 2008;132:311–22.
113 Vanhille L, Griffon A, Maqbool MA *et al.* High-throughput and quantitative assessment of enhancer activity in mammals by Capstarr-seq. *Nat Commun* 2015;6:6905.
114 Barski A, Cuddapah S, Cui K *et al.* High-resolution profiling of histone methylations in the human genome. *Cell* 2007;129:823–37.
115 Ziller MJ, Hansen KD, Meissner A, Aryee MJ. Coverage recommendations for methylation analysis by whole-genome bisulfite sequencing. *Nat Methods* 2015;12:230–2. 1 p following 32.
116 Birzele F, Fauti T, Stahl H *et al.* Next-generation insights into regulatory T cells: expression profiling and Foxp3 occupancy in human. *Nucleic Acids Res* 2011;39:7946–60.
117 Ranzani V, Rossetti G, Panzeri I *et al.* The long intergenic noncoding RNA landscape of human lymphocytes highlights the regulation of T cell differentiation by Linc-Maf-4. *Nat Immunol* 2015;16:318–25.
118 Zorca CE, Kim LK, Kim YJ *et al.* Myosin Vi regulates gene pairing and transcriptional pause release in T cells. *Proc Natl Acad Sci USA* 2015;112:E1587–93.

© 2017 The Foundation for the Scandinavian Journal of Immunology