CHAPTER 4

EPIGENETIC CONTROL IN IMMUNE FUNCTION

Peter J. van den Elsen,*[,1,2] Marja C.J.A. van Eggermond[1] and Rutger J. Wierda[1]

[1]*Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, Leiden, The Netherlands;* [2]*Department of Pathology, VU University Medical Center, Amsterdam, The Netherlands*

**Corresponding Author: Peter J. van den Elsen—Email: pjvdelsen@lumc.nl*

Abstract: This chapter describes recent advances in our understanding how epigenetic events control immune functions with emphasis on transcriptional regulation of *major histocompatibility complex Class I* (*MHC-I*) and *Class II* (*MHC-II*) genes. MHC-I and MHC-II molecules play an essential role in the adaptive immune response by virtue of their ability to present peptides, respectively to CD8+ and CD4+ T cells. Central to the onset of an adequate immune response to pathogens is the presentation of pathogen-derived peptides in the context of MHC-II molecules by antigen presenting cells (APCs) to CD4+ T cells of the immune system. In particular dendritic cells are highly specialized APCs that are capable to activate naïve T cells. Given their central role in adaptive immunity, *MHC-I* and *MHC-II* genes are regulated in a tight fashion at the transcriptional level to meet with local requirements of an effective antigen-specific immune response. In these regulatory processes the *MHC2TA* encoded Class II transactivator (CIITA) plays a crucial role. CIITA is essential for transcriptional activation of all *MHC-II* genes, whereas it plays an ancillary function in the transcriptional control of *MHC-I* genes. The focus of this chapter therefore will be on the transcription factors that interact with conserved cis-acting promoter elements and epigenetic mechanisms that modulate cell type-specific regulation of *MHC-I*, *MHC-II* and *MHC2TA* genes. Furthermore, we will also briefly discuss how genetic and epigenetic mechanisms contribute to T helper cell differentiation.

Epigenetic Contributions in Autoimmune Disease, edited by Esteban Ballestar.
©2011 Landes Bioscience and Springer Science+Business Media.

INTRODUCTION

The products of the *MHC Class I (MHC-I)* and *MHC Class II (MHC-II)* genes encode cell-surface glycoproteins involved in the binding and presentation of antigenic peptides to the T-cell receptors (TCRs) of T-lymphocytes. MHC-I proteins present peptides from endogenous sources, such as those derived from viruses, to $CD8^+$ T cells, whereas MHC-II molecules mainly present peptides from exogenous sources, such as those derived from extracellular pathogens, to $CD4^+$ T cells. These tri-molecular interactions of MHC, peptide and TCR are central to the generation of antigen-specific immune responses.

The *MHC-I* gene cluster encodes the highly polymorphic classical MHC-I molecules (Human Leukocyte Antigen (HLA)-A, -B and -C) and the less polymorphic nonclassical MHC-Ib molecules (HLA-E, -F and -G). Whereas the classical MHC-I molecules play essential roles in the detection and elimination of virus-infected cells, tumor cells and transplanted allogeneic cells, the MHC-Ib molecules have specialized immune regulatory functions (reviewed in 1). All cell surface expressed MHC-I and MHC-Ib molecules are associated with the nonpolymorphic β2-microglobulin. The MHC-II genes encode the polymorphic HLA-DR, -DQ and -DP molecules, which are expressed as α- and β-chain heterodimers on the cell surface. MHC-II molecules are central in the initiation of cellular and humoral immune responses, but they have also been implicated as contributing factors for a variety of autoimmune disorders. In contrast to MHC-I molecules, which are expressed in a constitutive fashion on almost all nucleated cells, the constitutive expression of MHC-II molecules is tissue-specific and is restricted to professional antigen presenting cells (APCs) of the immune system (reviewed in ref. 1). These APCs include dendritic cells, macrophages and B cells. All other cell types lack constitutive expression of MHC-II molecules, but their expression can be induced in an environment rich in inflammatory cytokines of which IFNγ is the most potent, or upon activation, such as in human T cells.[2] Because of their crucial role in the adaptive immune response, the genes encoding MHC-I and MHC-II molecules are tightly regulated by genetic and epigenetic mechanisms at the transcriptional level to provide an effective immune response against pathogens.

In its natural state, DNA is packaged into chromatin, a highly organized and dynamic protein-DNA complex, which consists of DNA, histones and nonhistone proteins. The fundamental subunit of chromatin, the nucleosome, is composed of an octamer of four core histones: two each of H2A, H2B, H3 and H4 surrounded by 146 bp of DNA.[3] Epigenetic changes are modifications in the architecture of chromatin without a change in the DNA sequence and determine the accessibility of chromatin. In this way, global gene activation and local control of gene-specific transcription is exerted by components of the epigenetic machinery. Methylation of DNA at CpG dinucleotides and modification or rearrangement of nucleosomes, which include covalent posttranslational modifications of histone tails, are key epigenetic chromatin marks.[4-6] Epigenetic modifications of histone tails include amongst others acetylation and methylation of lysine residues, phosphorylation of serine residues and methylation of arginine residues. The various histone modifications form a code which is read by nonhistone proteins and have varying effects on chromatin structure and gene accessibility.[4] As a rule of thumb, conformationally relaxed chromatin (euchromatin) is a hallmark of potentially active genes and is associated with hypomethylation of CpG dinucleotides in DNA and acetylated histones. Compact chromatin (heterochromatin) is associated with transcriptionally silent genes and is associated with DNA hypermethylation

at CpG dinucleotides and nonacetylated histones. The influence of histone methylation on gene expression depends on the exact lysine residue methylated and the number of added methyl groups.[7-11]

These chromatin modifications are exerted by epigenetic regulators such as DNA methyltransferases (DNMTs) and lysine acetyltransferases (KATs) and lysine methyltransferases (KMTs), which are increasingly being implicated as direct or indirect components in the regulation of expression of immune and other (tissue)-specific genes. Methylation and acetylation histone modifications are reversible. Lysine deacetylases (KDACs) and sirtuins (Sirt) remove acetylation modifications, whereas lysine demethylases (KDMs) remove methylation modifications.[12-15] In this way these enzymes promote a return to respectively repressive or active chromatin structure. In addition to this, histone and DNA modification activities are intimately linked. This is exemplified by the finding that triple methylated lysine 9 in histone H3 (H3K9me3) creates a binding platform for the various Heterochromatin Protein-1 (HP1) isoforms, which associate with Dnmt-1, Dnmt3a and Dnmt3b.[7,16-19] In addition, the KMTase Enhancer of Zeste Homologue-2 (EZH2 or KMT6), which trimethylates lysine 27 in histone H3 (H3K27me3), interacts with Dnmt's and in this way EZH2 recruits Dnmt activities to target promoters for CpG methylation.[20] As a result, these reversible epigenetic processes fine-tune gene expression patterns required for fundamental processes in the immune system such as cell activation, proliferation and differentiation. Moreover, it has also become apparent in recent years that many inflammatory disorders, including atherosclerosis, have an epigenetic component contributing to the disease.[21]

In the next paragraphs we will discuss the genetic and epigenetic mechanisms that direct transcriptional regulation of genes devoted to antigen presentation and to differentiation of T helper cells.

TRANSCRIPTIONAL REGULATION OF *MHC* GENES

Activation of *MHC-I* genes, with the exception of *HLA-G*, is mediated by several conserved regulatory elements within the various promoters: enhancer A, IFN-stimulated response element (ISRE) and the SXY-module (comprising the S, X1, X2 and Y-boxes). These conserved regulatory elements play an important role in the inducible and constitutive expression of *MHC-I* genes (reviewed in ref. 1). Of these regulatory elements, the SXY-module is also present is the promoters of *MHC-II* genes (Fig. 1). The sequence and stereo-specific alignment of the various boxes in the SXY-module is highly conserved and critical for its functioning in constitutive and inducible-transcriptional activation of *MHC-I* and *MHC-II* genes.[22,23] The SXY-module is cooperatively bound by a multi-protein complex containing regulatory factor X (RFX; consisting of RFX5, RFXB/ANK and RFXAP),[24-27] cyclic-AMP response element binding protein (CREB)/activating transcription factor (ATF)[22,28] and nuclear factor Y (NFY).[29,30] This complex acts as an enhanceosome driving transactivation of these genes.[22,31] In addition to these factors that assemble directly to the X1/X2 and Y box sequences, the co-activator CIITA (Class II transactivator) is also required. CIITA is essential for *MHC-II* transcription,[32] whilst it contributes to the activation of MHC-I promoters.[33] Given the essential role of CIITA in *MHC-II* transcription, constitutive expression of CIITA coincides with constitutive MHC-II molecule expression in APCs of which dendritic cells are the most efficient APCs. In all other types of cells

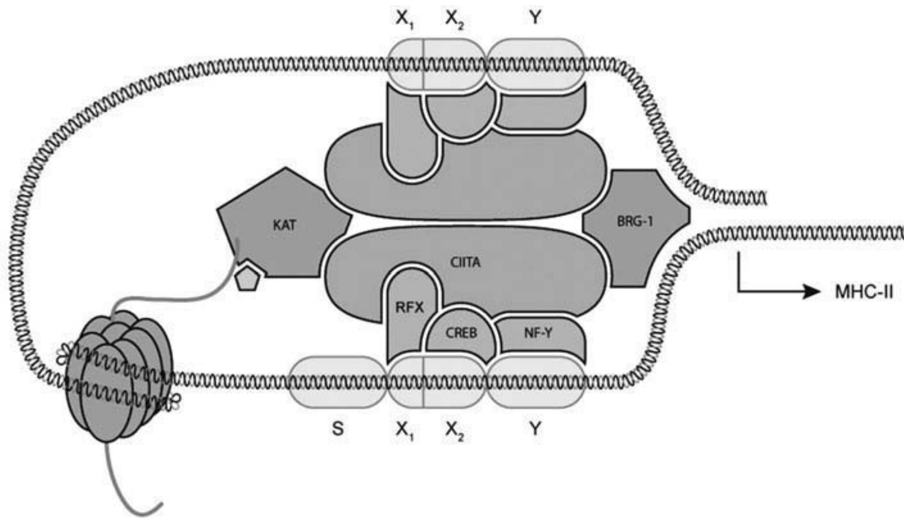

Figure 1. Schematic view of the factors and epigenetic events governing *MHC-II* transcription. Shown are the proximal SXY module and a distal XY element. The proximal SXY module is cooperatively bound by a multiprotein complex consisting of RFX (comprising RFXB/ANK, RFX5 and RFXAP), CREB/ATF and NFY. A similar binding pattern is observed for the more distal XY elements. CIITA interacts with the components of the multiprotein complex to positively regulate *MHC-II* transcription. CIITA recruits histone modification enzymes as detailed in the text, such as KATs and the general chromatin remodeler BRG1. CIITA has the capacity to self associate and this self association may allow bridging of the factors bound to the distal XY elements with the factors bound to the proximal SXY module to form a chromatin loop.

expression of CIITA can be induced by IFNγ resulting in inducible MHC-II expression at the cell surface. Thus CIITA acts as a molecular switch for MHC-II expression. Expression of *MHC-II* genes involves covalent modifications of histones and chromatin remodeling at the *MHC-II* genes. This is illustrated by the notion that IFNγ-induced MHC-II expression results in an increase in histone H3 and H4 acetylation in addition to an increase in the H4K4me3 modification at the MHC-II promoter, while a decrease in H3K9me3 was noted.[34]

LYSINE ACETYLTRANSFERASE/DEACETYLASE ACTIVITIES AND CIITA

CIITA exerts its transactivating function through protein-protein interactions with the components of the MHC-enhanceosome bound to the proximal SXY regulatory module in MHC promoters.[31,35,36] This interaction of CIITA with the MHC-enhanceosome allows for the subsequent association of CIITA with the KATs p300 (KAT3b)/CREB binding protein (CBP or KAT3a) and p300/CBP-associated factor (PCAF or KAT2b), which promote transcription of *MHC-I* and *MHC-II* genes by providing a more open chromatin structure.[22,23,37-39] Furthermore, CIITA also recruits the coactivator-associated arginine methyltransfease-1/protein arginine N-methyltransferase 4 (CARM1/PRMT4).[40,41] Besides acting as a platform for recruitment of KAT activities for transcriptional

control of *MHC-I* and *MHC-II* genes, CIITA itself contains intrinsic KAT activity.[42] CIITA-mediated transactivation of MHC promoters was found to rely on this intrinsic KAT activity, which maps to a region in its N-terminus.[42] This KAT activity of CIITA is regulated by its C-terminal GTP-binding domain and is stimulated by GTP.[42] Interestingly, the CIITA KAT activity was found to bypass TATA Box Binding Protein (TBP)-associated factor 250kD ($TAF_{II}250$) in MHC-I promoter activation.[42] Moreover, acetylation of CIITA itself by CBP and/or PCAF at specific lysine residues within the bipartite nuclear localization signal in the amino-terminal region of CIITA governs its nuclear accumulation.[39] As such these KATs control indirectly transcription of *MHC-I* and *MHC-II* genes.

In addition to KATs, CIITA also associates with lysine deacetylases (KDACs), which were found to interfere with CIITA function. KATs and KDACs thereby act as molecular switches for CIITA-mediated transcriptional activation/silencing of *MHC* genes. In this respect, it was found that KDAC1 and KDAC2 interfere in the transcriptional transactivation function of CIITA following IFNγ induction.[43,44] It has been shown in mice that the KDAC1/KDAC2-associated repressor SIN3 homolog A (mSin3A) amplifies this inhibition in CIITA function.[43] Endogenous CIITA and KDAC2 interact and KDAC2 has the potential to deacetylate CIITA in cultured cells.[44] As a result, CIITA is targeted to proteosomal degradation, which leads to a decreased interaction of CIITA with the RFX component RFX5 in a deacetylation dependent manner.[44] Together, these observations reveal that these KDAC activities affect CIITA function on the one hand by disrupting assembly of the MHC-enhanceosome, while on the other hand they interfere in CIITA interactions with the MHC-enhanceosome. The Switch/Sucrose NonFermentable (SWI/SNF) ATPase Brahma-related gene 1 (BRG-1) also associates with CIITA and is required for the CIITA-mediated induction of *MHC-II* genes.[45] The association of CIITA and BRG-1 suggest that the ATP-dependent chromatin remodeling SWI/SNF complex is recruited by CIITA to MHC-II promoters to control transcription of *MHC-II* genes.

Besides the crucial role of the proximal SXY-module in MHC-II promoters in the transcriptional regulation of *MHC-II* genes, the appropriate temporal and spacial expression of *MHC-II* genes in vivo also requires the involvement of additional, long-range regulatory elements. In these processes X-Y or X-box like sequences in the *MHC-II* region play an important role.[46] It has been found that interactions between the proximal elements and more distal X-Y or X-box like sequences (2.3 kb upstream of the HLA-DRA promoter) result in epigenetic changes at the MHC-II promoter.[47,48] In one model, RFX and CIITA can interact with the proximal SXY-module and with distal X-Y or X-box like sequences to form a chromatin loop.[47] Binding of CIITA to the distal X-box like sequences has been demonstrated by a chromatin looping technique.[47] This chromatin loop results in enhanced histone acetylation.[49] Likewise, the transcriptional insulator CCCTC binding factor (CTCF) was found to control *MHC-II* gene expression through long-distance chromatin interactions.[50] The intergenic DNA of the *HLA-DRB1* and *HLA-DQA1* genes hosts a region that was bound by CTCF and acts as a potent enhancer-blocking element.[51] This element and its bound factors was found to interact with *HLA-DRB1* and *HLA-DQA1* genes as determined in a quantitative 3C assay—an assay to detect long-distance chromatin interactions.[50] Subsequently it was demonstrated that CTCF associates with CIITA and RFX5 suggesting that the CTCF bound region and the flanking HLA-DRB1 and HLA-DQA1 proximal promoters may interact.[50]

EPIGENETIC REGULATION OF *MHC2TA* TRANSCRIPTION

As detailed above, CIITA is the 'master regulator' of MHC-II expression.[32] Transcriptional regulation of *MHC2TA*, the gene encoding CIITA, is mediated through the activity of four independent promoter units (CIITA-PI through CIITA-PIV) (Fig. 2A).[52] These promoter units are employed in a cell type- and activation-specific manner. CIITA-PI

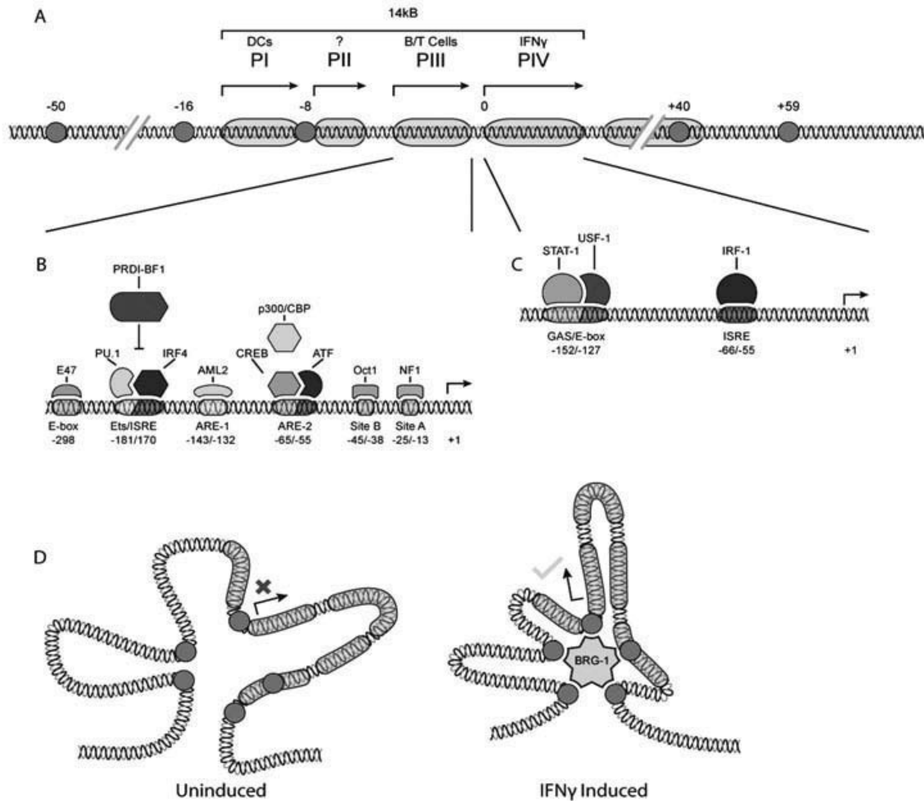

Figure 2. A) Schematic overview of the *MHC2TA* multipromoter region. Shown are the four MHC2TA promoters: CIITA-PI through CIITA-PIV. Grey spheres show BRG-1 binding sites relative to PIV. B) Factors and elements governing CIITA-PIII transactivation in B cells. Shown is the core promoter region of CIITA-PIII and the interacting factors. The localization of the various protein/DNA-binding elements is indicated relative to the transcription start site. Of these factors CREB/ATF has been shown to activate CIITA-PIII and this transactvation can be enhanced by p300/CBP. E47, PU.1 and IRF4 synergize to direct CIITA-PIII expression solely in B cells. C) Factors and elements governing CIITA-PIV transactivation following IFNγ stimulation. The localization of the various protein/DNA binding elements is indicated relative to the transcription start site. After stimulation with IFNγ, the IFNγ activated factor STAT1 binds directly together with USF1 to the GAS/E box motif in CIITA-PIV. Indirectly, STAT1 induces IRF1, which subsequently participates in the activation of CIITA-IV through binding to the ISRE. D) Model for chromatin loop formation of the *MHC2TA* multipromoter region after IFNγ stimulation involving BRG1-dependent distal enhancers (adapted from ref. 107). Grey spheres represent the relative locations of the BRG1-dependent distal enhancers, which interact weakly with each other and CIITA-PIV before IFNγ stimulation. These interactions are stabilized after IFNγ stimulation allowing transcription of the CIITA-PIV isoform.

and CIITA-PIII are used for the constitutive expression in dendritic cells and in B cells, respectively.[52] CIITA-PIV has been shown to be the promoter predominantly involved in IFNγ-inducible expression.[53-55] In addition, in human non-B cells, CIITA-PIII can also be activated by IFNγ through an element located 2 kb upstream of the core CIITA-PIII promoter.[55-57] CIITA-PIII has also been shown to be employed by human T cells upon activation.[2,58] The promoter function of CIITA-PII is still ill-defined. The various MHC2TA promoters each transcribe a unique first exon and are located within a region of approximately 14 kb.[52]

Several regulatory elements in *MHC2TA* promoters and interacting factors that are important for transcriptional activation have been identified. For induction of CIITA-PIV following stimulation with IFNγ, occupation of the GAS-box and the ISRE in CIITA-PIV by signal transducer and activator of transcription (STAT)-1 and the STAT-1 target gene interferon regulatory factor (IRF)-1 is crucial.[53] Furthermore interaction of STAT-1with upstream stimulatory factor (USF)-1 bound to the E-box adjacent to the GAS is required for stable interaction (Fig. 2C).[53] The IFNγ-mediated activation of CIITA-PIV also results in increased histone H3 and H4 acetylation at CIITA-PIV.[59] This increase in histone acetylation in CIITA-PIV chromatin is already noted prior to recruitment of IRF-1 to the CIITA-PIV promoter.[59] Interestingly, BRG-1 was also found to be an important factor in the IFNγ-mediated transcriptional activation of CIITA-PIV (which will be discussed further in the last paragraph of this section).[60] This notion is derived from studies with cells that lack expression of BRG-1, which failed to induce IFNγ-mediated CIITA expression.[60]

The transcription factor CREB-1 was found to play a key role in the activation of CIITA-PIII through its interaction with CRE-binding sites in the activation response element (ARE)-2 and, depending on the cellular context, in the 5′-UTR of CIITA-PIII.[2,56,58] The KAT CBP was shown to enhance CREB-1 mediated activation of CIITA-PIII in B cells.[56] CIITA-PIII also contains a composite PU.1/IRF-binding element (Site C) and 2 E-box motifs, which plays a crucial role in B-cell-specific transcriptional regulation of CIITA.[61] In B cells the Ets/ISRE-consensus element is bound by PU.1 and IRF-4, whereas the basic helix-loop-helix factor E47 interacts with the E-box motifs. PU.1, IRF-4 and E47 bound to the Ets/ISRE and E-boxes, respectively, synergize to direct B-cell-specific activation of CIITA-PIII (Fig. 2B).[61] This finding is of interest because PU.1, IRF-4 and E47 play an important role in B-cell differentiation and activation. These observations therefore provide a link between MHC-II mediated antigen presentation in B cells and B-cell differentiation and activation events.[61]

During B-cell differentiation to plasma cells, expression of CIITA is extinguished coinciding with loss of MHC-II cell surface expression.[62] This extinction of CIITA and resulting MHC-II molecule expression in plasma cells is mediated by the transcriptional repressor B lymphocyte-induced maturation protein 1 (Blimp-1, also known as Positive Regulatory Domain I-Binding Factor 1, PRDI-BF1).[63,64] The silencing of CIITA expression mediated by CIITA-PIII in plasma cells is most likely resulting from binding of Blimp-1 to the Ets/ISRE-consensus element (Site C) thereby disrupting the interaction of PU.1/IRF-4 to this element.[48,63,64]

Interestingly, besides its repressive activity on CIITA-PIII transactivation, there is also more recent evidence that PRDI-BF1 mediates also repression of CIITA-PIV.[65]

The fact that transcriptional repression by Blimp-1/PRDI-BF1 involves recruitment of KDACs, in particular KDAC1 and KDAC2 and the lysine methyltransferase KMT1C (also known as G9a), which catalyses dimethylation of lysine 9 in histone H3 (H3K9me2), this provides a strong link with epigenetic silencing of *MHC2TA* in plasma cells involving histone

acetylation and methylation modifications.[48,66,67] Indeed it was demonstrated by chromatin immunoprecipitation (ChIP) that differences in the levels of activating and repressive histone marks exists involving CIITA-PIII chromatin between B cells and plasma cells.[68] In plasma cells lacking CIITA expression, histone marks associated with gene transcription such as H3 and H4 acetylation and H3K4me2 and H3K4me3 are lost at CIITA-PIII, while the repressive H3K9me2 mark is increased.[68] Interestingly these histone marks were found also to exist at CIITA-PI, CIITA-PII and CIITA-PIV, revealing the involvement of the entire *MHC2TA* multipromoter region. As a consequence of the repressive histone marks and resulting chromatin inaccessibility, the binding of the CIITA-PIII interacting transcription factors (Sp-1, CREB-1, E47, PU.1, IRF-4) was lost in plasma cells.[68]

Chromatin remodeling also plays an important role in *MHC2TA* transcription in dendritic cell maturation.[69,70] Differentiation of monocytes into immature dendritic cells results in the induction of the CIITA-PI isoform, which directs expression of *MHC-II* genes. In immature dendritic cells, MHC-II molecules are largely retained in intracellular compartments. Upon maturation of dendritic cells, the peptide/MHC-II complexes are assembled and transported to the cell surface. During maturation of dendritic cells the increase of transported MHC-II molecules at the cell surface is accompanied by rapid transcriptional silencing of *MHC2TA* transcription.[69] The transcriptional inactivation of the *MHC2TA* multi-promoter locus is mediated by global histone deacetylation involving CIITA-PI, CIITA-PIII and CIITA-PIV[69]. Notably, during differentiation of monocytes into dendritic cells in a mouse model by mGM-CSF, activation of CIITA-PI is accompanied by an increase in histone H3 and H4 acetylation.[70] This increase in histone H3 and H4 acetylation was found to be blocked by IL-10, which resulted in inhibition of *MHC2TA* transcription.[70]

Distal elements and chromatin-remodeling also play an essential role in the transcriptional regulation of *MHC2TA*.[71] As mentioned before, *MHC2TA* has four alternative promoter units each transcribing its first exon (CIITA-PI through CIITA-PIV).[52] Transcriptional activation of the main IFNγ-responsive (CIITA-PIV) promoter was found to be dependent on the interaction with distal elements at -50kb and -8kb, which formed a loop with CIITA-PIV as determined by a chromatin conformation capture assay.[71] Contact was also detected between elements at -50kb and -16 kb. In these long-range interactions, BRG1, the ATPase driving the chromatin remodeling complex SWI-SNF (also called BAF), was constitutively bound to sites at -50kb, -16kb, -8kb and +59kb and also CIITA-IV as detailed above (Fig. 2D).[71] Thus BRG-1 not only is an important factor in the CIITA-mediated activation of MHC-II genes, but also controls the transcriptional activation of *MHC2TA* through long-range chromatin interactions and promoter interactions.

EPIGENETIC *MHC2TA* SILENCING IN CANCER

Downregulation of expression of MHC molecules is frequently noted in tumor cells. The low or lack of cell surface expression of both classes of MHC molecules impairs cellular immune recognition and resulting T-cell-mediated tumor eradication. Several studies have revealed that epigenetic modifications of chromatin play a critical role in the transcriptional silencing of *MHC2TA* and resulting extinction of *MHC-II* genes in cancer. In several cancer cell types, the lack of IFNγ-induced *MHC2TA* transcription is associated with CpG dinucleotide methylation of CIITA-PIV and also of CIITA-PIII DNA.[72-81] Besides CpG dinucleotide methylation, it has been suggested that the lack of

IFN-γ-induced transcription of *MHC2TA* in several cancer types is also associated with histone deacetylase activities.[82-86]

Of interest is the observation made in uveal melanoma tumor cell lines.[87] It was demonstrated that histone methylation played an important role in *MHC2TA* transcriptional silencing. The strongly reduced expression levels of CIITA after IFNγ-induction in an uveal melanoma cell line were found not to correlate with CpG dinucleotide methylation of CIITA-PIV DNA, but with high levels of the H3K27me3 histone modification in CIITA-PIV chromatin as determined by ChIP.[87] Consistent with the transcriptionally silent state of *MHC2TA* was the lack of RNA polymerase II recruitment into CIITA-PIV chromatin after IFNγ-induction in this cell line, while at the same time CIITA-PIV activating transcription factors were recruited.[87] RNA interference-mediated silencing of expression of the KMTase EZH2, resulted in an increment in CIITA mRNA expression levels after IFNγ induction. These observations suggest that EZH2 is involved in the transcriptional downregulation of IFNγ-induced expression of CIITA in uveal melanoma. Notably, the transcriptional silencing of *MHC2TA* by histone methylation in the absence of CpG dinucleotide methylation is in line with the observation that the H3K27me3 modification premarks genes for de novo methylation in cancer.[88] It could therefore be argued that the epigenetic make-up of the CIITA-PIV region in uveal melanoma reflects premarking for de novo methylation of DNA and that this reflects an intermediate epigenetic state of *MHC2TA* in the complete shut down of MHC-II mediated antigen presentation functions.

EPIGENETIC CONTROL OF T HELPER CELL DIFFERENTIATION

All T cells derive from the same precursor: the naïve T cell, which becomes activated after encounter of antigen in the context of MHC-II molecules at the cell surface of APC. After antigenic stimulation in the context of MHC-II, these naïve T cells can be differentiated into diverse T helper cell subsets, which include Th1, Th2, Th17 or $T_{reg.}$ In these differentiation processes specific cytokines and transcription factors, which determine lineage-specificity, play a critical role. In addition, these differentiation events are also regulated by epigenetic processes (for recent reviews see refs. 89-93). These epigenetic mechanisms are necessary to stably maintain gene expression patterns in the differentiated T helper cells and to eliminate the need for feedback loops.

The differentiation of naïve T cells into Th1 or Th2 is determined by the cytokines IL12 and IL4, respectively. In response to these signals, transcription is initiated of lineage specific cytokine genes including *IFN*γ and *IL4*.[92] The *IFN*γ and *IL4* loci are maintained in a 'poised' state in naïve T cells—i.e., they show both repressive and activating epigenetic marks—allowing rapid, early transcription. For instance, the IL4 promoter region exhibits a low basal level of histone H3 acetylation and DNA hypomethylation, but also shows repressive H3K27me3 modifications.[102] In Th1 cells expression of IFNγ is preceded by remodelling of the *IFN*γ locus.[94,95] Whereas in the differentiation to Th2 cells, IL-4 expression is preceded by remodelling of the *IL4* locus, similarly to the *IFN*γ locus remodelling in Th1 cells. Upon initial stimulation of naïve T cells, the lineage determining factors GATA3 and T-bet mediate many of the structural changes to the chromatin.[90,92] These factors will render the *IFN*γ or *IL4* genes, in respectively Th1 and Th2 cells, accessible to regulatory enzymes and other transcription factors.[96-98] This is illustrated by the notion that an increase in the level of expression of IFNγ was found in T cells from *Dnmt* knockout mice and in T cells treated with DNMT inhibitors.[99-101]

When naïve T cells are stimulated under Th1 conditions, transcription activating chromatin marks at the *IL4* locus are replaced with repressive marks, whereas the contrary happens under Th2 stimulating conditions (e.g., at the *IFNγ locus)*(reviewed in 103). Interestingly, differentiated T helper cells display an unconventional association of Polycomp Group (PcG) proteins.[104] Various members of the PcG family of proteins, including EZH2, bound to actively transcribed *IFNγ* and *IL4* genes in differentiating Th1 and Th2 cells.[104] This finding suggests that in addition to suppressing gene transcription, EZH2 might also act as a facilitator of gene transcription in T-lymphocyte differentiation. This might be achieved possibly through long-range interactions with distal regulatory elements.

The transcription factors T-bet and GATA3 are considered to be driving forces in Th1 or Th2 differentiation respectively. The Foxp3 transcription factor is considered the master switch for T_{reg}. The promoter of the Foxp3 transcription factor showed differences in methylation levels between T_{regs} and non-T_{reg} $CD4^+$ cells.[105] Furthermore, this study also showed differences in activating histone marks (H3Ac, H4Ac and H3K4me3) in Foxp3 promoter chromatin. Epigenetic regulation of T cell subtypes has also been shown in vivo. Mice which were treated with the KDAC inhibitor Trichostatin A showed an increase in $Foxp3^+$ $CD4^+$ T_{reg} cells in the lymphoid tissues.[106]

CONCLUSION

CIITA plays a central role in the control of constitutive and induced *MHC-II* gene transcription whereas it plays an ancillary function in constitutive and induced *MHC-I* gene transcription. The CIITA mediated transactivation of *MHC-II* and *MHC-I* genes is achieved through its interaction with the MHC-enhanceosome bound to the conserved SXY-module in MHC-II and MHC-I (with the exception of HLA-G) promoters. When bound to the MHC-enhanceosome, CIITA acts as a platform recruiting various activities involved in histone acetylation and deacetylation in the transcriptional control of *MHC* genes. Furthermore, CIITA is also central to recruitment of more general chromatin remodeling activities and long-range chromatin interactions of MHC-II promoters with distal elements. These activities mediated by CIITA provide tight control of transcription of these genes dedicated to antigen presentation. Moreover, the *MHC2TA* gene itself is tightly regulated at the transcriptional level by both genetic and epigenetic mechanisms. These include methylation of DNA and histone acetylation and methylation modifications. In addition, transcriptional activation of *MHC2TA* is mediated also through long-range chromatin interactions. Because of the involvement of epigenetic mechanisms in the transcriptional control of *MHC2TA* and T helper differentiation, deviations in these tightly regulated epigenetic mechanisms as observed under pathological conditions such as in cancer and autoimmune disease might provide an opportunity for pharmacological interference targeting the enzymes that modify DNA and histones.

ACKNOWLEDGEMENTS

Support of the research of PJvdE was obtained from the Dutch Cancer Society and the Dutch MS Research Foundation.

REFERENCES

1. Van den Elsen PJ, Holling TM, Kuipers HF et al. Transcriptional regulation of antigen presentation. Curr Opin Immunol 2004; 16:67-75.
2. Holling TM, Van der Stoep N, Quinten E et al. Activated human T-cells accomplish MHC class II expression through T-cell-specific occupation of class II transactivator promoter III. J Immunol 2002; 168:763-770.
3. Luger K, Mader AW, Richmond RK et al. Crystal structure of the nucleosome core particle at 2.8 A resolution. Nature 1997; 389:251-260.
4. Jenuwein T, Allis CD. Translating the histone code. Science 2001; 293:1074-1080.
5. Wu C. Chromatin remodeling and the control of gene expression. J Biol Chem 1997; 272:28171-28174.
6. Kuo MH, Allis CD. Roles of histone acetyltransferases and deacetylases in gene regulation. Bioessays 1998; 20:615-626.
7. Stewart MD, Li J, Wong J. Relationship between histone H3 lysine 9 methylation, transcription repression and heterochromatin protein 1 recruitment. Mol Cell Biol 2005; 25:2525-2538.
8. Cao R, Wang L, Wang H et al. Role of histone H3 lysine 27 methylation in Polycomb-group silencing. Science 2002; 298:1039-1043.
9. Ng HH, Ciccone DN, Morshead KB et al. Lysine-79 of histone H3 is hypomethylated at silenced loci in yeast and mammalian cells: a potential mechanism for position-effect variegation. Proc Natl Acad Sci USA 2003; 100:1820-1825.
10. Tachibana M, Sugimoto K, Fukushima T et al. Set domain-containing protein, G9a, is a novel lysine-preferring mammalian histone methyltransferase with hyperactivity and specific selectivity to lysines 9 and 27 of histone H3. J Biol Chem 2001; 276:25309-253.
11. Rice JC, Briggs SD, Ueberheide B et al. Histone methyltransferases direct different degrees of methylation to define distinct chromatin domains. Mol Cell 2003; 12:1591-1598.
12. De Ruijter AJ, Van Gennip AH, Caron HN et al. Histone deacetylases (HDACs): characterization of the classical HDAC family. Biochem J 2003; 370:737-749.
13. Holbert MA, Marmorstein R. Structure and activity of enzymes that remove histone modifications. Curr Opin Struct Biol 2005; 15:673-680.
14. Grozinger CM, Schreiber SL. Deacetylase enzymes: biological functions and the use of small-molecule inhibitors. Chem Biol 2002; 9:3-16.
15. Shi Y, Whetstine JR. Dynamic regulation of histone lysine methylation by demethylases. Mol Cell 2007; 25:1-14.
16. Lachner M, O'Carroll D, Rea S et al. Methylation of histone H3 lysine 9 creates a binding site for HP1 proteins. Nature 2001; 410:116-120.
17. Bannister AJ, Zegerman P, Partridge JF et al. Selective recognition of methylated lysine 9 on histone H3 by the HP1 chromo domain. Nature 2001; 410:120-124.
18. Fuks F, Hurd PJ, Deplus R et al. The DNA methyltransferases associate with HP1 and the SUV39H1 histone methyltransferase. Nucleic Acids Res 2003; 31:2305-2312.
19. Lehnertz B, Ueda Y, Derijck AA et al. Suv39h-mediated histone H3 lysine 9 methylation directs DNA methylation to major satellite repeats at pericentric heterochromatin. Curr Biol 2003; 13:1192-1200.
20. Vire E, Brenner C, Deplus R et al. The Polycomb group protein EZH2 directly controls DNA methylation. Nature 2006; 439:871-874.
21. Wierda RJ, Geutskens SB, Quax PHA et al. Epigenetics in atherosclerosis and inflammation. J Cell Mol Med 2010; in press.
22. Gobin SJP, Van Zutphen M, Westerheide SD et al. The MHC-specific enhanceosome and its role in MHC class I and beta(2)-microglobulin gene transactivation. J Immunol 2001; 167:5175-5184.
23. Ting JP, Trowsdale J. Genetic control of MHC class II expression. Cell 2002; 109:S21-33.
24. Steimle V, Durand B, Barras E et al. A novel DNA-binding regulatory factor is mutated in primary MHC class II deficiency (bare lymphocyte syndrome). Genes Dev 1995; 9:1021-1032.
25. Masternak K, Barras E, Zufferey M et al. A gene encoding a novel RFX-associated transactivator is mutated in the majority of MHC class II deficiency patients. Nat Genet 1998; 20:273-277.
26. Nagarajan UM, Louis-Plence P, DeSandro A et al. RFX-B is the gene responsible for the most common cause of the bare lymphocyte syndrome, an MHC class II immunodeficiency. Immunity 1999; 10:153-162.
27. Durand B, Sperisen P, Emery P et al. RFXAP, a novel subunit of the RFX DNA binding complex is mutated in MHC class II deficiency. EMBO J 1997; 16:1045-1055.
28. Moreno CS, Beresford GW, Louis-Plence P et al. CREB regulates MHC class II expression in a CIITA-dependent manner. Immunity 1999; 10:143-151.
29. Jabrane-Ferrat N, Nekrep N, Tosi G et al. Major histocompatibility complex class II transcriptional platform: assembly of nuclear factor Y and regulatory factor X (RFX) on DNA requires RFX5 dimers. Mol Cell Biol 2002; 22:5616-5625.

30. Louis-Plence P, Moreno CS, Boss JM. Formation of a regulatory factor X/X2 box-binding protein/nuclear factor-Y multiprotein complex on the conserved regulatory regions of HLA class II genes. J Immunol 1997; 159:3899-3909.
31. Masternak K, Muhlethaler-Mottet A, Villard J et al. CIITA is a transcriptional coactivator that is recruited to MHC class II promoters by multiple synergistic interactions with an enhanceosome complex. Genes Dev 2000; 14:1156-1166.
32. Steimle V, Otten LA, Zufferey M et al. Complementation cloning of an MHC class II transactivator mutated in hereditary MHC class II deficiency (or bare lymphocyte syndrome). Cell 1993; 75:135-146.
33. Gobin SJ, Peijnenburg A, Keijsers V et al. Site alpha is crucial for two routes of IFN gamma-induced MHC class I transactivation: the ISRE-mediated route and a novel pathway involving CIITA. Immunity 1997; 6:601-611.
34. Chou SD, Tomasi TB. Spatial distribution of histone methylation during MHC class II expression. Mol Immunol 2008; 45:971-980.
35. Zhu XS, Linhoff MW, Li G et al. Transcriptional scaffold: CIITA interacts with NF-Y, RFX and CREB to cause stereospecific regulation of the class II major histocompatibility complex promoter. Mol Cell Biol 2000; 20:6051-6061.
36. Jabrane-Ferrat N, Nekrep N, Tosi G et al. MHC class II enhanceosome: how is the class II transactivator recruited to DNA-bound activators? Int Immunol 2003; 15:467-475.
37. Kretsovali A, Agalioti T, Spilianakis C et al. Involvement of CREB binding protein in expression of major histocompatibility complex class II genes via interaction with the class II transactivator. Mol Cell Biol 1998; 18:6777-6783.
38. Fontes JD, Kanazawa S, Jean D et al. Interactions between the class II transactivator and CREB binding protein increase transcription of major histocompatibility complex class II genes. Mol Cell Biol 1999; 19: 941-947.
39. Spilianakis C, Papamatheakis J, Kretsovali A. Acetylation by PCAF enhances CIITA nuclear accumulation and transactivation of major histocompatibility complex class II genes. Mol Cell Biol 2000; 20:8489-8498.
40. Zika E, Fauquier L, Vandel L et al. Interplay among coactivator-associated arginine methyltransferase 1, CBP and CIITA in IFN-gamma-inducible MHC-II gene expression. Proc Natl Acad Sci USA 2005; 102:16321-16326.
41. Zika E, Ting JP. Epigenetic control of MHC-II: interplay between CIITA and histone-modifying enzymes. Curr Opin Immunol 2005; 17:58-64.
42. Raval A, Howcroft TK, Weissman JD et al. Transcriptional coactivator, CIITA, is an acetyltransferase that bypasses a promoter requirement for TAF(II)250. Mol Cell 2001; 7:105-115.
43. Zika E, Greer SF, Zhu XS et al. Histone deacetylase 1/mSin3A disrupts gamma interferon-induced CIITA function and major histocompatibility complex class II enhanceosome formation. Mol Cell Biol 2003; 23:3091-3102.
44. Kong X, Fang M, Li P et al. HDAC2 deacetylates class II transactivator and suppresses its activity in macrophages and smooth muscle cells. J Mol Cell Cardiol 2009; 46:292-299.
45. Mudhasani R, Fontes JD. The class II transactivator requires brahma-related gene 1 to activate transcription of major histocompatibility complex class II genes. Mol Cell Biol 2002; 22:5019-5026.
46. Gomez JA, Majumder P, Nagarajan UM et al. X box-like sequences in the MHC class II region maintain regulatory function. J Immunol 2005; 175:1030-1040.
47. Masternak K, Peyraud N, Krawczyk M et al. Chromatin remodeling and extragenic transcription at the MHC class II locus control region. Nat Immunol 2003; 4:132-137.
48. Wright KL, Ting JP. Epigenetic regulation of MHC-II and CIITA genes. Trends Immunol 2006; 27:405-412.
49. Krawczyk M, Peyraud N, Rybtsova N et al. Long distance control of MHC class II expression by multiple distal enhancers regulated by regulatory factor X complex and CIITA. J Immunol 2004; 173:6200-6210.
50. Majumder P, Gomez JA, Chadwick BP et al. The insulator factor CTCF controls MHC class II gene expression and is required for the formation of long-distance chromatin interactions. J Exp Med 2008; 205:785-798.
51. Majumder P, Gomez JA, Boss JM. The human major histocompatibility complex class II HLA-DRB1 and HLA-DQA1 genes are separated by a CTCF-binding enhancer-blocking element. J Biol Chem 2006; 281:18435-18443.
52. Muhlethaler-Mottet A, Otten LA, Steimle V et al. Expression of MHC class II molecules in different cellular and functional compartments is controlled by differential usage of multiple promoters of the transactivator CIITA. EMBO J 1997; 16:2851-2860.
53. Muhlethaler-Mottet A, Di Berardino W, Otten LA et al. Activation of the MHC class II transactivator CIITA by interferon-gamma requires cooperative interaction between Stat1 and USF-1. Immunity 1998; 8:157-166.

54. Piskurich JF, Wang Y, Linhoff MW et al. Identification of distinct regions of 5′ flanking DNA that mediate constitutive, IFN-gamma, STAT1 and TGF-beta-regulated expression of the class II transactivator gene. J Immunol 1998; 160:233-240.
55. Piskurich JF, Linhoff MW, Wang Y et al. Two distinct gamma interferon-inducible promoters of the major histocompatibility complex class II transactivator gene are differentially regulated by STAT1, interferon regulatory factor 1 and transforming growth factor beta. Mol Cell Biol 1999; 19:431-440.
56. Van der Stoep N, Quinten E, Van den Elsen PJ. Transcriptional regulation of the MHC class II trans-activator (CIITA) promoter III: identification of a novel regulatory region in the 5′-untranslated region and an important role for cAMP-responsive element binding protein 1 and activating transcription factor-1 in CIITA-promoter III transcriptional activation in B lymphocytes. J Immunol 2002; 169:5061-5071.
57. Van der Stoep N, Quinten E, Alblas G et al. Constitutive and IFNgamma-induced activation of MHC2TA promoter type III in human melanoma cell lines is governed by separate regulatory elements within the PIII upstream regulatory region. Mol Immunol 2007; 44:2036-2046.
58. Wong AW, Ghosh N, McKinnon KP et al. Regulation and specificity of MHC2TA promoter usage in human primary T-lymphocytes and cell line. J Immunol 2002; 169:3112-3119.
59. Morris AC, Beresford GW, Mooney MR et al. Kinetics of a gamma interferon response: expression and assembly of CIITA promoter IV and inhibition by methylation. Mol Cell Biol 2002; 22:4781-4791.
60. Pattenden SG, Klose R, Karaskov E et al. Interferon-gamma-induced chromatin remodeling at the CIITA locus is BRG1 dependent. EMBO J 2002; 21:1978-1986.
61. Van der Stoep N, Quinten E, Marcondes Rezende M et al. E47, IRF-4 and PU.1 synergize to induce B-cell-specific activation of the class II transactivator promoter III (CIITA-PIII). Blood 2004; 104:2849-2857.
62. Silacci P, Mottet A, Steimle V et al. Developmental extinction of major histocompatibility complex class II gene expression in plasmocytes is mediated by silencing of the transactivator gene CIITA. J Exp Med 1994; 180:1329-1336.
63. Piskurich JF, Lin KI, Lin Y et al. BLIMP-I mediates extinction of major histocompatibility class II transactivator expression in plasma cells. Nat Immunol 2000; 1:526-532.
64. Ghosh N, Gyory I, Wright G et al. Positive regulatory domain I binding factor 1 silences class II transactivator expression in multiple myeloma cells. J Biol Chem 2001; 276:15264-15268.
65. Chen H, Gilbert CA, Hudson JA et al. Positive regulatory domain I-binding factor 1 mediates repression of the MHC class II transactivator (CIITA) type IV promoter. Mol Immunol 2007; 44:1461-1470.
66. Yu J, Angelin-Duclos C, Greenwood J et al. Transcriptional repression by blimp-1 (PRDI-BF1) involves recruitment of histone deacetylase. Mol Cell Biol 2000; 20:2592-2603.
67. Gyory I, Wu J, Fejér G et al. PRDI-BF1 recruits the histone H3 methyltransferase G9a in transcriptional silencing. Nat Immunol 2004; 5:299-308.
68. Green MR, Yoon H, Boss JM. Epigenetic regulation during B-cell differentiation controls CIITA promoter accessibility. J Immunol 2006; 177:3865-3873.
69. Landmann S, Muhlethaler-Mottet A, Bernasconi L et al. Maturation of dendritic cells is accompanied by rapid transcriptional silencing of class II transactivator (CIITA) expression. J Exp Med 2001; 194:379-391.
70. Choi YE, Yu HN, Yoon CH et al. Tumor-mediated down-regulation of MHC class II in DC development is attributable to the epigenetic control of the CIITA type I promoter. Eur J Immunol 2009; 39:858-868.
71. Ni Z, Abou El HM, Xu Z et al. The chromatin-remodeling enzyme BRG1 coordinates CIITA induction through many interdependent distal enhancers. Nat Immunol 2008; 9:785-793.
72. Morris AC, Spangler WE, Boss JM. Methylation of class II trans-activator promoter IV: a novel mechanism of MHC class II gene control. J Immunol 2000; 164:4143-4149.
73. Van den Elsen PJ, Van der Stoep N, Viëtor HE et al. Lack of CIITA expression is central to the absence of antigen presentation functions of trophoblast cells and is caused by methylation of the IFN-gamma inducible promoter (PIV) of CIITA. Hum Immunol 2000; 61:850-862.
74. Meissner M, Whiteside TL, Van Kuik-Romein P et al. Loss of interferon-gamma inducibility of the MHC class II antigen processing pathway in head and neck cancer: evidence for posttranscriptional as well as epigenetic regulation. Br J Dermatol 2008; 158:930-940.
75. Holling TM, Van Eggermond MC, Jager MJ et al. Epigenetic silencing of MHC2TA transcription in cancer. Biochem Pharmacol 2006; 72:1570-1576.
76. Van den Elsen PJ, Holling TM, Van der Stoep N et al. DNA methylation and expression of major histocompatibility complex class I and class II transactivator genes in human developmental tumor cells and in T-cell malignancies. Clin Immunol 2003; 109:46-52.
77. Holling TM, Schooten E, Langerak AW et al. Regulation of MHC class II expression in human T-cell malignancies. Blood. 2004; 103:1438-1444.
78. Van der Stoep N, Biesta P, Quinten E et al. Lack of IFN-gamma-mediated induction of the class II transactivator (CIITA) through promoter methylation is predominantly found in developmental tumor cell lines. Int J Cancer 2002; 97:501-507.

79. De Lerma Barbaro A, De Ambrosis A, Banelli B et al. Methylation of CIITA promoter IV causes loss of HLA-II inducibility by IFN-gamma in promyelocytic cells. Int Immunol. 2008; 20:1457-1466.
80. Satoh A, Toyota M, Ikeda H et al. Epigenetic inactivation of class II transactivator (CIITA) is associated with the absence of interferon-gamma-induced HLA-DR expression in colorectal and gastric cancer cells. Oncogene 2004; 23:8876-8886.
81. Morimoto Y, Toyota M, Satoh A et al. Inactivation of class II transactivator by DNA methylation and histone deacetylation associated with absence of HLA-DR induction by interferon-gamma in haematopoietic tumour cells. Br J Cancer 2004;90: 844-852.
82. Chou SD, Khan AN, Magner WJ et al. Histone acetylation regulates the cell type specific CIITA promoters, MHC class II expression and antigen presentation in tumor cells. Int Immunol. 2005; 17:1483-1494.
83. Murphy SP, Holtz R, Lewandowski N et al. DNA alkylating agents alleviate silencing of class II transactivator gene expression in L1210 lymphoma cells. J Immunol 2002; 169:3085-3093.
84. Magner WJ, Kazim AL, Stewart C et al. Activation of MHC class I, II and CD40 gene expression by histone deacetylase inhibitors. J Immunol 2000; 165:7017-7024.
85. Kanaseki T, Ikeda H, Takamura Y et al. Histone deacetylation, but not hypermethylation, modifies class II transactivator and MHC class II gene expression in squamous cell carcinomas. J Immunol 2003; 170:4980-4985.
86. Holtz R, Choi JC, Petroff MG et al. Class II transactivator (CIITA) promoter methylation does not correlate with silencing of CIITA transcription in trophoblasts. Biol Reprod 2003; 69:915-924.
87. Holling TM, Bergevoet MW, Wilson L et al. A role for EZH2 in silencing of IFN-gamma inducible MHC2TA transcription in uveal melanoma. J Immunol 2007; 179:5317-5325.
88. Schlesinger Y, Straussman R, Keshet I et al. Polycomb-mediated methylation on Lys27 of histone H3 premarks genes for de novo methylation in cancer. Nat Genet 2007; 39:232-236.
89. Lee CG, Sahoo A, Im SH. Epigenetic regulation of cytokine gene expression in T-lymphocytes. Yonsei Med J 2009; 50:322-330.
90. Wilson CB, Rowell E, Sekimata M. Epigenetic control of T-helper-cell differentiation. Nat Rev Immunol 2009; 9:91-105.
91. Placek K, Coffre M, Maiella S et al. Genetic and epigenetic networks controlling T helper 1 cell differentiation. Immunology 2009; 127:155-162.
92. Amsen D, Spilianakis CG, Flavell RA. How are T(H)1 and T(H)2 effector cells made? Curr Opin Immunol 2009; 21:153-160.
93. Lee YK, Mukasa R, Hatton RD et al. Developmental plasticity of Th17 and Treg cells. Curr Opin Immunol 2009; 21:274-280.
94. Schoenborn JR, Wilson CB. Regulation of interferon-gamma during innate and adaptive immune responses. Adv Immunol 2007; 96:41-101.
95. Avni O, Lee D, Macian F et al. T(H) cell differentiation is accompanied by dynamic changes in histone acetylation of cytokine genes. Nat Immunol 2002; 3:643-651.
96. Ansel KM, Lee DU, Rao A. An epigenetic view of helper T-cell differentiation. Nat Immunol 2003; 4:616-623.
97. Murphy KM, Reiner SL. The lineage decisions of helper T-cells. Nat Rev Immunol 2002; 2:933-944.
98. Rao A, Avni O. Molecular aspects of T-cell differentiation. Br Med Bull 2000; 56:969-984.
99. Makar KW, Wilson CB. DNA methylation is a nonredundant repressor of the Th2 effector program. J Immunol 2004; 173:4402-4406.
100. Young HA, Dray JF, Farrar WL. Expression of transfected human interferon-gamma DNA: evidence for cell-specific regulation. J Immunol 1986; 136:4700-4703.
101. Young HA, Ghosh P, Ye J et al. Differentiation of the T helper phenotypes by analysis of the methylation state of the IFN-gamma gene. J Immunol 1994; 153:3603-3610.
102. Koyanagi M, Baguet A, Martens J et al. EZH2 and histone 3 trimethyl lysine 27 associated with Il4 and Il13 gene silencing in Th1 cells. J Biol Chem 2005; 280:31470-31477.
103. Ansel KM, Djuretic I, Tanasa B et al. Regulation of Th2 differentiation and Il4 locus accessibility. Annu Rev Immunol 2006; 24:607-656.
104. Jacob E, Hod-Dvorai R, Schif-Zuck S et al. Unconventional association of the polycomb group proteins with cytokine genes in differentiated T helper cells. J Biol Chem 2008; 283:13471-13481.
105. Floess S, Freyer J, Siewert C et al. Epigenetic control of the foxp3 locus in regulatory T-cells. PLoS Biol 2007; 5:e38.
106. Tao R, de Zoeten EF, Ozkaynak E et al. Deacetylase inhibition promotes the generation and function of regulatory T-cells. Nat Med 2007; 13:1299-307.
107. Reith W, Boss JM. New dimensions of CIITA. Nat Immunol 2008; 9:713-714.