REGULATION OF ANTIGEN PRESENTATION IN THE IMMUNE SYSTEM

Walter Reith

The bodies of humans and other animals constitute a vast and appealing resource-rich milieu in which foreign microorganisms can live, feed, reproduce and shelter from the harsh and hostile external environment. We are therefore continuously subjected to onslaughts by a wide variety of potentially pathogenic organisms, including myriads of different viruses, bacteria, fungi, and unicellular and multicellular parasites. To combat the establishment and propagation of infections we are equipped with a complex network of molecules, cells and tissues collectively referred to as the immune system. Our immune system is specialized for recognizing and alerting us to the presence of foreign organisms, for mounting efficacious and multifaceted immune responses that are tailored for combating and eliminating diverse infectious agents, and for providing long-lasting immunity preventing the recurrence of previously encountered infections. Because of these key functions, the immune system is essential for survival. In the absence of a fully functional immune system – as is for instance the case in hereditary and acquired immunodeficiency diseases, or following the administration of immunosuppressive drugs to treat autoimmune diseases, avoid transplant rejection or attenuate graft versus host disease – even minor infections can take hold and prove fatal.

In parallel to fighting and clearing infections, our immune system also plays a key role in recognizing and eliminating tumors. Conversely, it must remain tolerant to healthy self-tissues, and avoid mounting unwanted immune responses directed against therapeutic drugs or innocuous foreign molecules, such as those found in ingested nutrients, plant pollen or cat dander. The loss of self-tolerance, and the inability to avoid vigorous reactions against medicaments, nutrients or harmless substances, lead to pathological immune responses and hypersensitivity reactions that underpin autoimmune diseases and allergies.

The initiation and development of protective immune responses directed against infectious agents and tumors in the context of an immune system that remains self-tolerant and avoids hyper-reactivity to beneficial or harmless compounds is strictly dependent on the ability to recognize and distinguish between self or innocuous antigens and foreign antigens derived from infectious organisms. Major Histocompatibility Complex (MHC) molecules play a central role in this process because they serve as cell-surface display cases specialized for presenting peptide antigens to the immune system.

Expression and function of MHC class II molecules

T-lymphocytes constitute a population of diverse cell types implicated at various levels in the initiation, propagation and regulation of protective immune responses directed against infectious agents and tumors, as well as in the maintenance of self-tolerance. Most major types of T-cells carry a receptor – called the T-cell receptor (TCR) – that is dedicated for the recognition of peptide antigens bound to MHC molecules expressed at the surface of other cell types. Of prime importance for appropriate functioning of the immune system in health and disease are T-cells carrying the CD4 co-receptor. The TCRs of $CD4^+$ T-cells recognize peptides presented by MHC class II (MHCII) molecules displayed at the surface of specialized cells of the immune system (**Figure 1**). Peptides presented by MHCII molecules are derived mainly from extracellular or membrane-bound proteins that have been internalized and degraded in endosomal/lysosomal compartments.

Key MHCII-positive cells include thymic epithelial cells (TEC) in the cortical (cTEC) and medullary (mTEC) compartments of the thymus, and cells that are specialized for the capture and presentation of antigens, collectively referred to as professional antigen presenting cells (APC) (**Figure 1**) (reviewed in 1–4). APC include B-lymphocytes, cells belonging to the monocyte-macrophage lineage, and various dendritic cell (DC) subtypes. Within these cell lineages, MHCII expression is fine-tuned as a function of developmental stage, activation status, interactions with other cells, and/or exposure to extracellular stimuli in-

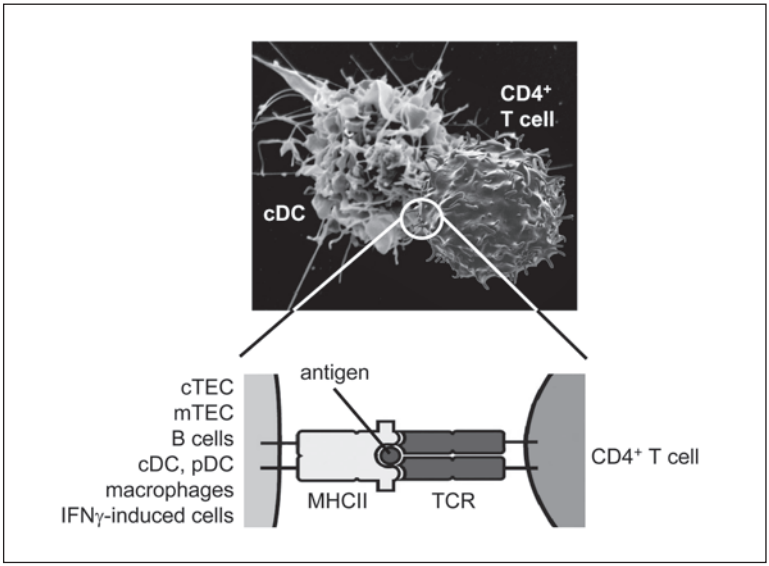

Figure 1. *Expression and function of MHCII molecules. MHCII molecules are heterodimeric transmembrane glycoproteins proteins expressed at the surface of specialized cells of the immune system, including cortical thymic epithelial cells (cTEC), medullary thymic epithelial cells (mTEC), B-cells, various different types of dendritic cell including conventional DC (cDC) and plasmacytoid DC (pDC), and cells belonging to the monocyte-macrophage lineage. MHCII expression is also induced on other cell types when they are stimulated with IFNγ. MHCII molecules present peptides to the antigen receptor (TCR) of CD4⁺ T-cells.*

cluding cytokines, inflammatory signals and microbial compounds (1–4). Most other cell types – such as fibroblasts, endothelial cells and epithelial cells in various tissues – generally lack constitutive MHCII expression. However, the majority of these MHCII-negative cells can be induced to activate MHCII expression by exposure to interferon-γ (IFNγ) (1–4), thereby licensing them to participate as «non-professional» APC during ongoing immune responses.

MHCII-mediated antigen presentation is essential for several key processes in the immune system. MHCII expression in the thymus guides

the development of $CD4^+$ thymocytes and shapes the antigen-specificity of their TCR repertoire. MHCII-positive cTEC drive positive selection (survival) of $CD4^+$ thymocytes carrying TCR capable of interacting with self MHCII molecules. MHCII-positive mTEC and DC in the medulla establish self-tolerance by promoting the negative selection (deletion) of autoreactive $CD4^+$ thymocytes carrying TCR specific for self-antigens. Thymic MHCII expression is also implicated in the selection of regulatory $CD4^+$ T-cells (Treg), which are critical for maintaining peripheral tolerance by inhibiting autoreactive T-cells that have evaded negative selection in the thymus. In the periphery, MHCII-mediated antigen presentation by APC controls $CD4^+$ T-cell activation and thus orchestrates the initiation, regulation and development of antigen-specific immune responses directed against pathogens and tumors, as well as against self-antigens during the course of autoimmune diseases. MHCII-mediated antigen presentation by DC plays a dual role. Under steady-state non-inflammatory conditions, immature DC help to maintain peripheral tolerance. Conversely, following activation by infections and/or inflammatory signals, DC adopt a mature phenotype that converts them into potent APC for the activation of naïve T-cells and the initiation of immune responses. MHCII expression by B-cells allows them to collaborate with $CD4^+$ T-cells for the generation of high-affinity antibody responses. Finally, MHCII expression by macrophages is critical for their ability to receive help from $CD4^+$ T-cells for the elimination of intracellular pathogens.

Regulation of MHCII expression; lessons from a severe hereditary immunodeficiency syndrome

The immunological processes implicating MHCII-mediated antigen presentation all depend on a strict cell-type specific and quantitatively controlled pattern of MHCII expression. Deficient or inappropriate MHCII expression therefore has profound pathological consequences. This has prompted us and others to engage in an intensive and long-lasting research effort aimed at understanding the molecular mechanisms that regulate the expression of MHCII genes (reviewed in 1–6).

MHCII molecules are heterodimeric (α/β chain) transmembrane glycoproteins. In man there are three MHCII isotypes (HLA-DR, -DQ and –DP) while in mice there are only two (H-2A and H-2E). Intracellular routing and peptide loading of MHCII molecules is controlled by their association with an accessory protein called the invariant chain (Ii) and two non-classical MHCII-like molecules called HLA-DM (H-2M in mice) and HLA-DO (H-2O in mice). The α and β chain genes of all classical and non-classical MHCII molecules are clustered together in the genome, in the class II region of the MHC locus. Expression of the MHCII, Ii and HLA-DM (H-2M) genes is tightly coregulated. HLA-DO (H-2O) expression is also coregulated, albeit in a more cell type restricted pattern limited to B-cells, mTEC and certain DC subtypes. Coordinate expression is controlled at the level of transcription by a highly-conserved regulatory module (S-Y module) found in the promoter of each gene (**Figure 2**) (1–6). The S-Y module is a transcriptional enhancer consisting of four conserved cis-acting DNA sequences – known as the S, X, X2 and Y boxes – present in a tightly constrained order, orientation and spacing (**Figure 2**) (1–6).

The molecular machinery that controls MHCII expression via the S–Y module was unraveled to a large extent by the study of a hereditary immunodeficiency disease called MHCII-deficiency or the Bare Lymphocyte Syndrome (reviewed in 1, 6–8). MHCII-deficiency is caused by a virtually complete absence of MHCII expression. The loss of MHCII expression leads to defective $CD4^+$ T-cell development in the thymus, the inability to present antigens to $CD4^+$ T-cells in the periphery, and thus to dramatically impaired immune responses (1, 7, 8). Patients consequently exhibit a severe susceptibility to infections, primarily of the respiratory and gastrointestinal tracts, by a wide range of infectious agents, including viruses, bacteria, fungi and parasites (1, 7, 8). These infections generally lead to failure to thrive and death in early childhood.

MHCII-deficiency is due to mutations in regulatory genes required for MHCII expression (reviewed in 1, 6–8). Patients have been classified into four complementation groups corresponding to defects in four regulatory genes. Our past work contributed directly or indirectly to identification of the genes affected in the four MHCII-deficiency com-

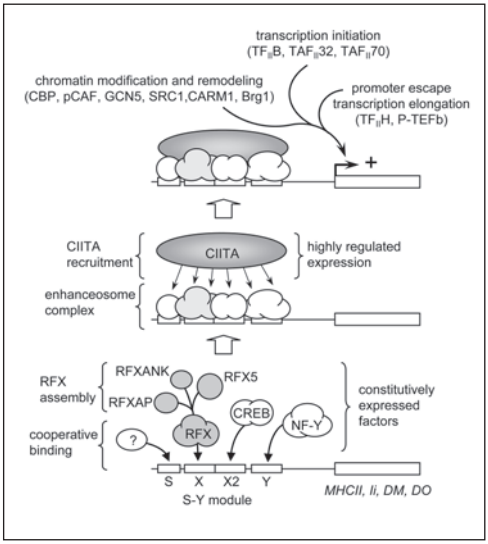

Figure 2. *Overview of MHCII gene regulation. The ubiquitously expressed factors RFX, CREB, and NF-Y bind cooperatively to generate a highly stable enhanceosome complex on the S-Y modules found in the promoters of MHCII, Ii, DO and DM genes (bottom). This enhanceosome complex serves as a platform to which the highly regulated non-DNA-binding coactivator CIITA is recruited (middle) by means of multiple protein-protein interactions (arrows). Mutations in the genes coding for the shaded factors (CIITA, RFX5, RFXANK and RFXAP) give rise to the MHCII-deficiency disease. The enhanceosome complex and CIITA collaborate in promoting chromatin remodeling by recruiting histone acetyltransferases, histone methyltransferases and nucleosome remodeling factors (top). They are also implicated in the recruitment of components of the general transcription initiation machinery, and factors involved in promoter clearance and transcription elongation (top). Abbreviations: Brg1, Brahma-related gene 1; CARM1 coactivator-associated arginine methyltransferase 1; CBP, CREB-binding protein; CREB, cAMP response element-binding protein; GCN5, general control of amino acid synthesis 5; NF-Y, nuclear factor Y; pCAF, p300/CBP-associated factor; P-TEFb, positive transcription elongation factor b; SRC1, steroid receptor coactivator 1; TAF, TBP-associated factor; TBP, TATA-box-binding protein; $TF_{II}B$ and $TF_{II}H$, general transcription factors for RNA Polymerase II.*

plementation groups (9–12). Isolation of these genes led to the identification of two critical MHCII-specific transcription factors called Regulatory Factor X (RFX) and the Class II Transactivator (CIITA). RFX is a DNA-binding transcription factor that binds to the X box of the S–Y module and consists of three essential subunits called RFX5, RFXAP and RFXANK (also called RFX-B [6]) (**Figure 2**). CIITA is a non-DNA-binding transcriptional coactivator that is recruited to MHCII promoters via protein-protein interactions with RFX and other transcription factors (CREB, NF-Y) bound to the S-Y module (**Figure 2**) (1, 5–8).

To date all MHCII-deficiency patients can be accounted for by complete or partial loss-of-function mutations in the genes encoding RFX5, RFXAP, RFXANK or CIITA (1, 6–8). Both CIITA and RFX are therefore essential components of the molecular machinery that regulates the coordinate transcription of MHCII genes. The key role of CIITA and RFX in the regulation of MHCII expression was also confirmed by the generation of *CIIta*$^{-/-}$ and *Rfx5*$^{-/-}$ mice.

Given their pivotal roles as regulators of MHCII gene transcription, our work has for many years concentrated on elucidating the structure, function, mode of action and specificity of RFX and CIITA, and on studying how these factors regulate the complex cell-type-specific and inducible pattern of MHCII expression (4, 7, 13). Furthermore, we have exploited the knowledge gained by these studies to generate transgenic and knockout mouse models that have been invaluable for deciphering the role of MHCII expression by specific cell types *in vivo* in the context of an intact immune system (4, 13).

Structure, function and mode of action of RFX and CIITA

RFX5, the largest subunit of the RFX complex, derives its name from the fact that it was the 5th identified mammalian member of the RFX family of transcription factors (10, 14). RFX factors serve a diverse range of functions in species ranging from yeast to man (14). They all bind to X box related sequences via a conserved DNA-binding domain. Establishment of the structure of the DNA-binding domain of RFX1 demonstrated that it is related to the winged-helix subfamily of helix-turn-helix domains (15). Structure-function analysis demonstrated that the DNA-binding-domain region of RFX5 is sufficient for its association with RFXAP and RFXANK, and thus for the assembly of a functional RFX complex capable of activating MHCII expression (16).

An animal model for MHCII-deficiency has been generated by gene targeting of the mouse *Rfx5* gene (17). *Rfx5*$^{-/-}$ mice reproduce all major phenotypical and immunological features of the human disease, including the loss of MHCII expression, impaired positive selection of

$CD4^+$ T-cells, decreased peripheral $CD4^+$ T-cell counts and a deficiency in $CD4^+$ T-cell dependent immune responses (17).

All mammalian RFX factors can bind to the X box of MHCII promoters *in vitro*. However, only RFX5 is required for MHCII expression *in vivo*. The other family members have very different functions. The generation of *Rfx3*$^{-/-}$ mice has for instance allowed us to demonstrate that RFX3 is required for the formation and function of cilia on various cell types (18, 19), and for the development and function of pancreatic endocrine cells (19).

RFXAP (RFX associated protein), the second subunit of RFX, derives its name from the fact that it associates with RFX5 but does not contain the characteristic DNA-binding domain of the RFX family (11). RFXAP exhibits no homology to any other human proteins. Structure-function analysis demonstrated that a glutamine-rich C-terminal region of RFXAP is essential and sufficient for its association with RFX5 and RFXANK, assembly of the RFX complex and activation of MHCII genes (20). We also defined a domain that is required for optimal expression of HLA-DP and HLA-DQ but dispensable for HLA-DR (20).

RFXANK, the smallest subunit of RFX, derives its name from the fact that it contains a domain consisting of four tandemly repeated «ankyrin» motifs (12, 21). Ankyrin repeat domains (ARD) are well-known protein-protein interaction domains found in numerous different proteins. Structure-function analysis has demonstrated that the ARD of RFXANK is essential and sufficient for assembly of the RFX complex and for activation of MHCII genes (21). Critical sub-domains mapping to four distinct surfaces of the three-dimensional structure of the ARD were defined by a series of single and clustered point mutations (21). Three of these surfaces mediate protein-protein contacts with RFXAP and RFX5. The fourth serves an unknown function required for MHCII promoter occupation by RFX and CIITA *in vivo*.

The trimeric RFX complex binds to the X box cooperatively with the X2-box-binding factor CREB and the Y-box-binding factor NF-Y (6, 7). This generates a stable multi-protein complex called the MHCII

«enhanceosome» (**Figure 2**) (22). An as yet unidentified S-box-binding factor also assembles into the enhanceosome complex (23). The well-defined architecture of the S-Y module reflects spatial constraints imposed by assembly of the enhanceosome. The enhanceosome constitutes a landing pad to which CIITA is recruited (5–7, 22). In RFX-deficient cells, enhanceosome assembly and CIITA recruitment are abolished, such that MHCII promoters remain completely unoccupied *in vivo* (7).

CIITA is a non-DNA-binding transcriptional coactivator that is recruited to the MHCII enhanceosome by means of multiple protein-protein interactions (**Figure 2**) (5–7, 22). Protein-protein contacts have been defined between CIITA and various enhanceosome components, including two subunits of RFX (RFXANK, RFX5), two subunits of NF-Y (NF-YB, NF-YC), CREB and the S-box-binding factor (5–7). These multiple contacts are believed to function synergistically to promote stable recruitment of CIITA to the MHCII enhanceosome complex.

The enhanceosome and CIITA collaborate to activate transcription of MHCII genes by promoting the establishment of histone modifications, inducing chromatin remodeling, facilitating recruitment of the general transcription machinery (GTM), activating promoter clearance by RNA polymerase II (Pol II) and stimulating transcription elongation (**Figure 2**) (5–7, 24). Most studies have suggested that these effector mechanisms are mediated mainly by CIITA. CIITA has been proposed to activate transcription by recruiting the coactivator OBF1, histone acetyltransferases (CBP, pCAF, GCN5, SRC1), histone methyltransferases (CARM1), nucleosome remodeling factors (Brg1), components of the GTM ($TF_{II}B$, $TF_{II}D$) and transcription elongation factors ($TF_{II}H$, P-TEFb) (5–7, 24). CIITA may also harbor an intrinsic acetyltransferase domain (5–7, 24). The relative importance of these different activities of CIITA remains unclear.

In contrast to CIITA, a more passive role limited to the formation of a docking surface for CIITA has generally been attributed to the enhanceosome complex. However, there is growing evidence that the functional importance of CIITA has been overemphasized whereas that of the

enhanceosome complex has been underestimated. We have now shown that several functions initially attributed to CIITA (such as the introduction of histone H3 acetylation and methylation) are in fact secondary consequences of ongoing transcription (25). We have also demonstrated that the enhanceosome makes critical CIITA-independent contributions to histone acetylation and to recruitment of the GTM and Pol II at certain MHCII promoters (*HLA-DPB and HLA-DMB*) (26). More recently, we have shown that the enhanceosome plays a critical role in inducing nucleosome eviction from MHCII promoters (27). This nucleosome eviction unmasks the transcription start site and thereby ensures that transcription is initiated at the correct position (27). Finally, RFX and the enhanceosome complex protect MHCII promoters against the establishment of epigenetic silencing by DNA methylation (28), thereby ensuring that MHCII genes remain accessible and inducible in all cell types, even in MHCII-negative cells lacking CIITA.

In addition to acting locally at MHCII promoters, the enhanceosome and CIITA also function at distant S–Y (S'–Y') modules scattered throughout the MHCII locus (29, 30). Binding of the enhanceosome and CIITA to these S'–Y' enhancers induces long-distance chromatin remodeling as evidenced by a global increase in histone acetylation (29, 30). It also induces the synthesis of intergenic transcripts of unknown function (29, 30). Both features are typical of locus control regions (LCR), which are regulatory regions exerting long-distance control over gene expression. Unpublished results have indicated that the distal S'–Y' modules engage in long-distance chromatin interactions with each other, with MHCII promoters, and/or with other regulatory sequences. Collectively, these findings suggest that the regulation of MHCII promoters by RFX and CIITA may be superimposed by global control mechanisms exerted over all or large segments of the MHCII locus by distant regulatory elements.

Target gene specificity of RFX and CIITA

The phenotype of MHCII-deficiency patients had initially suggested that CIITA and RFX are highly specific for MHCII genes. It was sub-

sequently demonstrated that these factors also regulate the expression of accessory genes required for MHCII-mediated antigen presentation, namely the Ii gene and the genes coding for the non-classical MHCII molecules HLA-DO (H-2O) and HLA-DM (H-2M) (5–7). Additional studies revealed that these factors also contribute, albeit to a lesser degree, to the expression of MHC class I (MHCI) genes (5–7). For many years, these genes remained the only well-documented targets of CIITA and RFX, and these factors were therefore believed to be highly dedicated for genes implicated in antigen presentation.

The high specificity of CIITA for genes involved in antigen presentation has been challenged by several studies suggesting that it regulates numerous other genes within and outside of the immune system, including the IL-4, FasL, Collagen α2(I), thymidine kinase, cyclin D1 and plexin A1 genes, as well as numerous genes revealed by microarray experiments performed with CIITA-deficient B-cells (reviewed in 4, 13). However, the regulation of these genes by CIITA has in most cases turned out to be indirect, relatively minor, mediated by unknown mechanisms, or controversial (4, 13). Using CIITA transgenic and knockout mice (31), as well as genome-wide bioinformatic and ChIP-chip (chromatin immunoprecipitation coupled to microarray) screens (30, 32, 33), we have notably been unable to confirm for most of the proposed target genes that they are indeed regulated directly by CIITA.

To settle the controversial issue of the target-gene specificity of CIITA we developed two genome-wide approaches designed to identify *bona-fide* new CIITA-regulated genes. First, we developed a computational approach to search the genome for the presence of new S–Y modules (30, 32). Second, we performed genome-wide ChIP-chip experiments to identify new target genes of CIITA in human B-cells and DC (33). These combined approaches demonstrated that CIITA is remarkably dedicated for the regulation of genes implicated in MHCII and MHCI mediated antigen presentation. Only very few novel target genes were identified, several of which could be implicated directly or indirectly in antigen presentation (32, 33). We have concentrated on studying the regulation of two of these genes, *RAB4B* and *BTN2A2*.

RAB4B (*Rab4b* in mice) encodes a member of the Rab family of small GTPases, which regulate intracellular vesicular-transport processes. The biological function of RAB4B/Rab4b is unknown. However, the closely related Rab4a functions in early endosome sorting and endosome recycling. Expression of a dominant negative variant of Rab4a in B-cells was found to interfere with receptor-mediated antigen-processing and MHCII-mediated antigen presentation. We have confirmed that *RAB4B* expression is indeed regulated by both RFX and CIITA in human B- cells, DC and IFNγ-induced cells (32).

The *BTN2A2* gene (*Btn2a2* in mice) encodes a member of a family of cell-surface proteins sharing homology to butyrophilin (BTN). BTN is required for regulated secretion of milk-lipid droplets. The functions of BTN-like proteins remain largely unknown. However, they are homologous to the B7 family of immunoregulatory proteins, and there is growing evidence that they have key functions in the immune system. We have discovered that the *BTN2A2/Btn2* gene is tightly coregulated with MHCII genes by RFX and CIITA in human and mouse B-cells, DC, IFNγ-induced cells and TEC (unpublished data), raising the exciting possibility that BTN2A2/Btn2a2 may serve a key immunoregulatory function associated with MHCII-mediated antigen presentation.

Regulation of CIITA expression

Whereas RFX and the other enhanceosome factors exhibit widespread expression in most cell types, the expression of CIITA is tightly regulated in a cell-type specific and inducible manner (4–7, 13). It is therefore the expression pattern of the gene encoding CIITA that determines the cell-type specificity and level of MHCII expression. We have made major contributions to our current understanding of this pivotal function of CIITA as the master regulator of MHCII genes.

Expression of the human *CIITA* gene is directed by four independent promoters – called pI, pII, pIII, and pIV – which precede four alternative first exons (**Figure 3**) (4-7, 13). Three of these promoters – pI, pIII and pIV – are conserved in the mouse *CIIta* gene (**Figure 3**) (4–7, 13).

The three conserved *CIITA/CIIta* promoters differ in their cell type specificity and response to IFNγ (4–7, 13). It is thus the differential activity of the *CIITA/CIIta* promoters that ultimately determines the cell type specificity and inducibility of MHCII gene expression (4–7, 13).

The functions of the *CIITA/CIIta* promoters have been defined by examining their usage in cell lines and key primary human and mouse cell types, by mapping regulatory elements using *in vivo* footprint experiments, transcription factor binding studies and functional assays, and by generating knockout mice in which the promoters were excised singly or in combination (4–7, 13). We notably constructed mice lacking pI (pI$^{-/-}$ mice, unpublished data), pIV (pIV$^{-/-}$ mice) (34, 35), and both pIII and pIV (pIII+IV$^{-/-}$ mice) (36). Collectively, these and other studies have generated a precise picture of the function and specificity of the three *CIITA/CIIta* promoters (**Figure 3**) (4–7, 13).

Promoter pIV is essential for driving CIITA and MHCII expression in cTEC and in IFNγ induced cells of non-hematopoietic origin (**Figure 3**) (34, 35). Activation in cTEC is a pivotal function of pIV because MHCII expression by cTEC is essential for driving positive selection of CD4^{+} thymocytes. Positive selection of CD4^{+} thymocytes is completely abolished in pIV$^{-/-}$ mice (34, 35). The IFNγ-induced activity of pIV is targeted by several signals that inhibit the expression of MHCII genes (**Figure 3**) (4–7, 13). The repression of MHCII induction in trophoblasts is due to silencing of pIV. Anti-inflammatory cytokines capable of inhibiting IFNγ-induced MHCII expression – such as TGFβ, IL4 and IL10 – achieve this by inhibiting the activation of pIV.

pIII is the major promoter used in B-cells (**Figure 3**) (36). Silencing of pIII following B-cell-activation is responsible for the extinction of MHCII expression in plasma cells (**Figure 3**) (4-7, 13). pIII is also essential for driving CIITA and MHCII expression in a unique DC subtype called plasmacytoid DC (pDC) (**Figure 3**) (36). This recently allowed us to exploit our pIII+IV$^{-/-}$ mice to demonstrate that MHCII-mediated antigen presentation by pDC induces the development of Treg conferring protection against the development of experimental autoim-

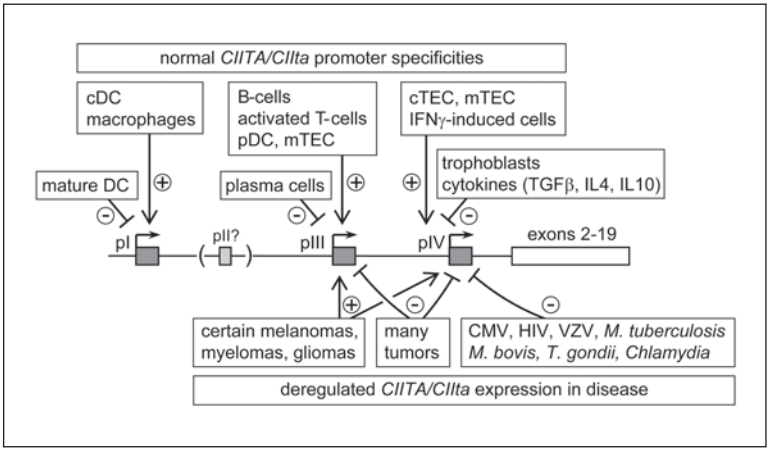

Figure 3. *Regulation of CIITA expression. Transcription of the human* ***CIITA*** *gene is controlled by four independent promoters (pI, pII, pIII, and pIV) preceding four alternative first exons (grey boxes). Three of these promoters (pI, pIII and pIV) are conserved in the mouse* ***CIIta*** *gene. The three conserved promoters exhibit well-defined cell-type specificities and responsiveness to IFNγ. The function of pII is not known. Promoter pI is active mainly in macrophages and conventional dendritic cells (cDC). Promoter pIII is active in B-cells, activated human T-cells, plasmacytoid dendritic cells (pDC) and medullar thymic epithelial cells (mTEC). Promoter pIV is active in both cortical and medullar thymic epithelial cells (cTEC, mTEC), and is induced by IFNγ in cells of non-hematopoietic origin. Promoters pI, pIII and pIV are silenced in mature DC, plasma cells, and trophobasts, respectively. Activation of pIV is inhibited by certain anti-inflammatory cytokines. Aberrant activation or silencing of pIII and pIV is observed in various tumors and infectious diseases.*

mune encephalomyelitis (EAE) (unpublished data), which is an autoimmune disease of the central nervous system (CNS) that is widely studied as an animal model for multiple sclerosis.

Promoter pI mediates CIITA and MHCII expression in macrophages and all conventional DC (cDC) subsets examined, including human monocyte-derived DC (MoDC), mouse bone-marrow-derived DC (BmDC), epidermal Langerhans cells, and splenic, lymph node and thymic cDC (**Figure 3**) (36). This has allowed us to exploit our $pI^{-/-}$ mice to demonstrate that the effector phase of EAE in the CNS is sup-

ported by collaboration between MHCII-mediated antigen presentation by blood-derived APC (cDC, macrophages) and CNS-derived APC (microglia, microglia-derived DC) (unpublished data).

Transcription of the *CIITA/CIIta* gene is silenced during the maturation of all cDC subsets studied (human MoDC, mouse BmDC, splenic DC) (**Figure 3**) (37). This silencing process is triggered by a wide range of stimuli, including lipopolysacharide (LPS), tumor necrosis factor α (TNFα, CD40 ligand (CD40L), interferon α (IFNα), bacterial and viral infections (37). Silencing is essentially complete within 1 hour, and is mediated by a global repression mechanism implicating histone deacetylation over a large domain spanning the entire regulatory region of the *CIITA* gene (unpublished data). We have recently performed a genome-wide ChIP-chip analysis of histone deacetylation induced in human MoDC by a brief 1 hour exposure to LPS. The results of this analysis suggest that the rapid epigenetic silencing mechanism we have documented for *CIITA* also impinges on numerous other genes in cDC, and is thus likely to have widespread importance for the cDC maturation process (unpublished data).

In contrast to cTEC, mTEC rely on both pIII and pIV for driving CIITA and MHCII expression (**Figure 3**) (38). This difference between cTEC and mTEC with respect to *CIIta* promoter usage has recently allowed us to exploit our pIII+IV$^{-/-}$ mice, in combination with a series of other transgenic and knockout mouse models, to demonstrate that MHCII expression by mTEC is required for the generation of mature mTEC (38). Briefly, we discovered that the development of wild type numbers of mature mTEC is dependent on TCR/MHCII-mediated interactions between thymocytes carrying autoreactive TCR and mTEC displaying the cognate self-antigens in the context of MHCII molecules (38). Mature mTEC play pivotal roles in the establishment and maintenance of T-cell tolerance because they are implicated in both the deletion of autoreactive thymocytes and the development of tolerizing Treg cells. The role of autoreactive CD4^{+} thymocytes and their interactions with MHCII-positive mTEC in the development and function of the thymic medulla are therefore being pursued actively.

Finally, it is becoming increasingly evident that deregulation of the *CIITA* promoters is associated with diseases (**Figure 3**) (reviewed in 4, 13). Both silencing and aberrant activation of promoters pIII and pIV have been associated with various types of tumors (4, 13). Furthermore, a variety of pathogens have developed the ability to inhibit IFNγ-induced activation of pIV expression – and thus MHCII expression – as a strategy to evade recognition by the immune system (4, 13). Notable examples include CMV, HIV, varicella-zoster virus, *Mycobacterium bovis, Mycobacterium tuberculosis, Toxoplasma gondii*, and *Chlamydia*. Finally, there is growing evidence that polymorphisms in the promoters of the *CIITA* gene could be associated with autoimmune and inflammatory diseases.

Conclusions and Perspectives

A great deal has been learned about the molecular machinery that regulates the expression of MHCII genes. However, this progress has also uncovered numerous unresolved issues and paved the way for challenging new avenues of research. The following are among the key questions that remain to be addressed by future work. Is the regulation of MHCII promoters by RFX and CIITA superimposed by global control mechanisms exerted over all or large segments of the MHCII locus? Do these global regulatory mechanisms involve a three-dimensional structure established by long-distance chromatin interactions? What is the nature and specificity of the regulatory mechanisms that impinge on the CIITA gene in key APC such as DC? Are the mechanisms that trigger rapid silencing of the *CIITA* gene in DC of more widespread importance for the maturation process, and what functions or pathways do these mechanisms regulate? Do the newly identified target genes of RFX and CIITA contribute to APC function and/or other functions in the immune system? How is the immune system perturbed by selective abrogation of CIITA expression in key cell types, such as mTEC? How does deregulated *CIITA* promoter function contribute to the development off disease? Addressing such questions will continue to be a major objective of our future work, because we hope that the information that will be gained will further our comprehension of the importance of

correctly regulated MHCII-expression for appropriate functioning of the immune system in healthy individuals, and will shed new light on the mechanisms and pathological consequences of deregulated MHCII expression during the course of diseases such as cancer, autoimmune pathologies and infectious diseases.

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Due to lack of space I have restricted myself mainly to citing suitable comprehensive reviews on the subject of MHC class II gene regulation, and key original research papers from my own laboratory. This by no means denotes a lack of acknowledgement on my part of the large number of significant contributions made by other research groups. I sincerely apologize for being unable to cite their work and encourage the reader to examine the original literature referred to in the review articles cited here.

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