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Biochemical Pharmacology 68 (2004) 1209–1213
Biochemical Pharmacology
www.elsevier.com/locate/biochempharm

Epigenetic control of CIITA expression in leukemic T cells

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Received 23 February 2004; accepted 26 March 2004

Keywords: CIITA; T cells; Leukemia; Lymphoma; DNA methylation; Histone acetylation; Chromatin

1. Introduction

The products of the major histocompatibility complex (MHC) class II genes encode cell surface glycoproteins involved in binding and presentation of antigenic peptides from exogenous sources to the T cell receptors of $CD4^+$ T lymphocytes. These trimolecular interactions of MHC/peptide-TCR are central to the initiation of antigen-specific immune responses. MHC class II genes encode the polymorphic HLA-DR, -DQ and -DP proteins, which are expressed as αβ heterodimers on the cell surface. Because of their specialized immunological role, constitutive expression of MHC class II proteins is confined to antigen presenting cells (APC), which include dendritic cells (DCs), macrophages, B-lymphocytes and thymic epithelial cells. On all other cell types expression of MHC class II molecules can be induced in an environment rich in inflammatory cytokines, of which interferon-γ (IFNγ) is the most potent, or upon activation such as in human T cells [1,2].

Besides their important role in the regulation of the immune response, MHC class II molecules can also serve as signal transducing receptors [3]. Particularly, in lymphocytes it has been well documented that ligation of MHC class II molecules results in activation of intracellular signaling pathways [2]. For instance in activated T lymphocytes triggering of MHC class II molecules results in protein kinase C (PKC) membrane translocation and inositol phosphate (IP) accumulation, while simultaneous signaling via MHC class II molecules and CD3 results in a synergistic effect on IP accumulation correlating with significantly increased CD3-mediated T blast proliferation [4]. In addition, signaling through MHC class II molecules has also been implicated on the one hand in induction of caspase-independent cell death while on the other hand the MHC class II-mediated signaling route interferes with FAS-mediated apoptosis in B-lymphocytes [5–7]. Recently, an engineered human monoclonal antibody against the HLA-DR molecule was shown to have a tumoricidal activity on $HLA\text{-}DR^+$ hematopoietic tumors [8], revealing a putative potent role of MHC class II-induced cell death in targeted antibody therapy against MHC class II bearing tumor cells [9].

2. Transcriptional regulation of MHC class II gene expression

The various MHC class II genes contain the so-called SXY regulatory module in their promoters, a set of four conserved sequence motifs: the S or W box, the X1 box, the X2 box, and the Y box [10]. The SXY-module is cooperatively bound by a multiprotein complex containing RFX (consisting of RFX5, RFXB/ANK and RFXAP), CREB/ATF, and NFY, which acts as an enhanceosome driving transactivation of these genes (Fig. 1). In addition to the above factors that assemble directly with the X and Y box

Abbreviations: CIITA, class II transactivator; MHC, major histocompatibility complex; HLA, human leukocyte antigens; APC, antigen presenting cell; DC, dendritic cell; HAT, histone acetyltransferase; HDAC, histone deacetylase; CBP, CREB-binding protein; PCAF, p300/CBP-associated factor; ChIP, chromatin immunoprecipitation; RFX, regulatory factor X; CREB, cAMP response element binding protein; NFY, nuclear factor Y; IFN, interferon; PKC, protein kinase C; IP, inositol phosphate; BLS, bare lymphocyte syndrome; ATF, activating transcription factor; BRG, Brahma-related gene; ARE, activation response element; AML, acute myeloid leukaemia; IVGF, in vivo genomic footprint

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0006-2952/$ – see front matter
doi:10.1016/j.bcp.2004.03.046

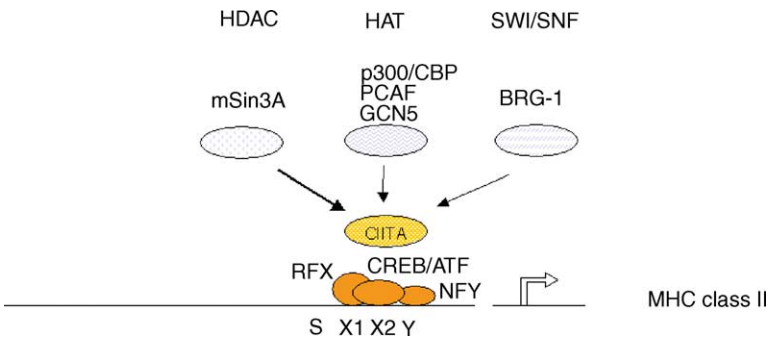

Fig. 1. Factors governing MHC class II expression. Shown is the conserved SXY module, which is bound by the MHC-enhanceosome comprising RFX, CREB/ATF and NFY. CIITA binds to the MHC-enhanceosome and recruits HAT and ATP-dependent chromatin remodelers for gene transcription or HDAC for gene silencing.

sequences, the class II transactivator (CIITA) is also required. CIITA is essential for MHC class II expression [11] and exerts its transactivation function through protein/protein interactions with individual components of the MHC-enhanceosome [12–14]. The crucial importance of RFX and CIITA in MHC class II gene regulation is illustrated by studies with cell lines established from patients with the bare lymphocyte syndrome (BLS), a severe combined immunodeficiency due to mutations in one of the RFX subunits or CIITA [15]. Lack of either CIITA or one of the RFX subunits affects respectively the functioning and assembly of the MHC-enhanceosome, leading to a lack of MHC class II expression.

CIITA recruits various histone acetyltransferases (HATs) into the MHC-enhanceosome (i.e. p300/CBP, GCN5 and P/CAF; Fig. 1) [16–18]. On the one hand this HAT activity is important for histone tail modifications and resulting remodelling of chromatin structure, while on the other hand CIITA itself is acetylated which promotes CIITA activity [19]. Moreover, CIITA itself contains an intrinsic HAT activity [20]. In addition to HATs, ATP-dependent chromatin remodelling factors such as the Brahma-related gene 1 (BRG-1), a component of the SWI/SNF complex, cooperate in CIITA-mediated activities (Fig. 1) [21,22]. BRG-1 plays an important role in the IFNγ-mediated transcriptional activation of the CIITA gene, but BRG-1 is also recruited by the CIITA protein to MHC class II promoters for their activation [21,22].

While HATs promote CIITA function in the transactivation of MHC class II genes, histone deacetylases (HDACs) [23] interfere with this CIITA function. HDAC-1 and -2 inhibit the transactivation function of CIITA following IFNγ-induction, which is intensified by the activity of mSin3A, an HDAC-1, -2-associated repressor. These HDACs affect CIITA function in two ways: through interference in MHC-enhanceosome assembly and through interference in CIITA interactions with components of the MHC-enhanceosome. CIITA therefore may integrate HAT or HDAC activities thereby acting as a molecular switch to modulate transcription of its target genes in the MHC class II antigen presentation pathway.

3. Factors and elements that control expression of CIITA in T cells

As described above, CIITA is of crucial importance for the transcriptional regulation of MHC class II genes. Therefore, coinciding with MHC class II expression, the constitutive expression of CIITA is confined to APCs only, and CIITA expression can be induced in various other cell types. The transcriptional regulation of human CIITA is controlled by at least three independent promoter units (CIITA-PI, CIITA-PIII and CIITA-PIV) each transcribing a unique first exon [24]. CIITA-PIII is utilized for the constitutive expression of CIITA in the hematopoietic lineage while CIITA-PI is utilized in DC only (N. Van der Stoep, E. Quinten, P.J. Van den Elsen, unpublished observations) [24,31]. CIITA-PIV has been shown to be the promoter predominantly involved in IFNγ-inducible CIITA expression in non-bone marrow derived cells [25,26]. In addition to CIITA-PIV, CIITA-PIII is also induced by IFNγ induction in human cells [27,28]. Recently it was established that normal human activated T cells exclusively employ CIITA-PIII to drive expression of CIITA [29,30]. However, the factors and elements that contribute to CIITA-PIII activation in the various cell types within the lymphoid lineage is still under investigation.

In activated T cells the activation response element (ARE)-1 and -2, and site-A, -B and -C are occupied as determined by in vivo genomic footprint (IVGF) analyses [29]. Similar to the previously established role of these elements in Raji B cells, the ARE-1 and ARE-2 were found essential for CIITA-PIII activity in T cell lines [29,32]. The ARE-1 element was shown to bind both the Runx family members AML2 and AML3 in T cells, while in B cells only AML2 binds to this promoter region [29]. The ARE-2 site was found to bind CREB/ATF family members and both CREB-1 and ATF-1 contributed to CIITA-PIII activation in vitro, which was further upregulated by the general co-activator CBP [28,30]. More importantly, CREB-1 was found to be associated with chromatin at CIITA-PIII as determined by chromatin immunoprecipitation (ChIP) assay [28].

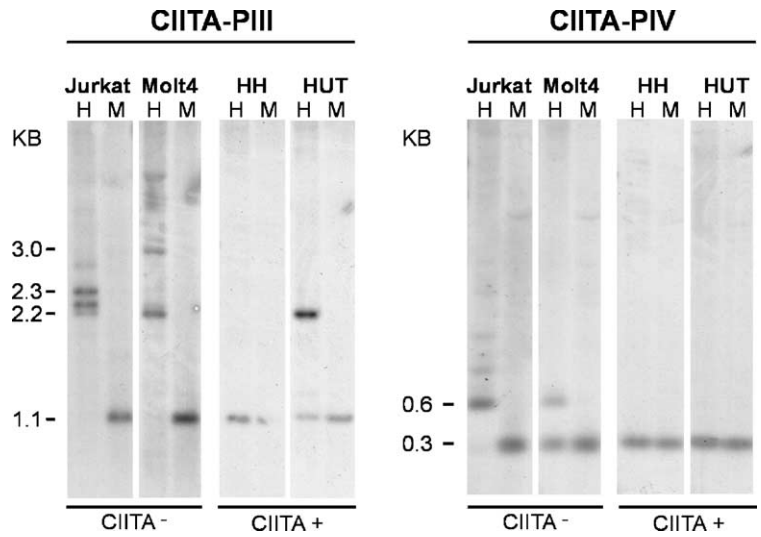

Fig. 2. Southern blot analysis revealing hypermethylation at CIITA-PIII and CIITA-PIV in CIITA-deficient T leukemic cell lines. Shown are the digests with Hpa II (which does not cleave at 5′-CC^{Me}GG-3′) and its isoschizomer MspI (which cleaves methylated and unmethylated DNA). For details see [33].

4. Epigenic control of CIITA in leukemic T cells

Leukemic T cell lines and freshly isolated leukemic T cells lack expression MHC class II molecules, which is the result from lack of CIITA expression [33]. This is corroborated by the notion that Jurkat leukemic T cells re-express MHC class II on the cell surface following introduction of exogenous CIITA [34]. Furthermore, the CIITA-deficiency of leukemic T cell lines is not resulting from absence of transcription factors critical for CIITA-PIII activation because a CIITA-PIII reporter is activated in transient transfection assays to similar levels compared with CIITA-expressing lymphoma cell lines [33]. The CIITA-deficiency of leukemic T cell lines and freshly isolated leukemic T cells correlates with hypermethylation of CIITA-PIII and also CIITA-PIV [33]. This is illustrated in Fig. 2 in which we have analysed CpG island methylation patterns involving CIITA-PIII and CIITA-PIV in a Southern blot assay with genomic DNA from CIITA expressing lymphoma cells and CIITA-deficient leukemic cell lines. Digestion of genomic CIITA-PIII and CIITA–PIV DNA from Jurkat and Molt-4 CIITA-deficient leukemic T cell lines with the methylation sensitive restriction enzyme Hpa II and the methylation tolerant restriction enzyme MspI shows digestion of CIITA-PIII and CIITA-PIV DNA by MspI only. Both enzymes cut the DNA in the CIITA positive lymphoma cell lines. Treatment with the demethylating agent 5-AZA-2′-deoxycytidine resulted in re-expression of CIITA-PIII, but not CIITA-PIV, and expression of HLA-DRA in the leukemic T cell lines (Fig. 3A) [33].

We next investigated whether reduction in the amount of acetylated histone H3 and H4 tails correlated with the CIITA-deficient phenotype of the leukemic T cell lines. Using chromatin immunoprecipitation (ChIP) the association of Ac-H4 with CIITA-PIII chromatin in Jurkat T cells was less prominent when compared with CIITA-PIII chromatin in CIITA expressing Raji B cells (Fig. 3B). Similar results were obtained for Ac-H3 (results not shown). To determine the involvement of an HDAC-dependent transcriptional repression mechanism targeted at CIITA-PIII and thereby contributing to the CIITA-deficient phenotype

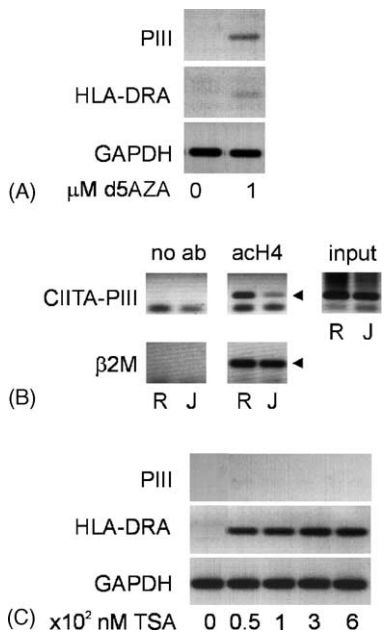

Fig. 3. (A) 5′-AZA-2′-deoxycytidine treatment of Jurkat CIITA-deficient leukemic T cells restores CIITA and HLA-DRA expression (for experimental details see [33]). (B) Chromatin immunoprecipitation using anti-Ac-H4 (Upstate Cell Signaling 06-866) revealing reduced amounts of Ac-H4 associated with CIITA-PIII chromatin. The β2-microglobulin chromatin IP was used as control (for experimental details see [29]). (C) Trichostatin A (TSA) treatment of Jurkat leukemic T cells revealing lack of CIITA induction in Jurkat T cells as determined by RT-PCR (for experimental details on the RT-PCR see [33]). Note the induction of HLA-DRA upon TSA mediated inhibition of HDAC activities in the absence of CIITA.

of Jurkat T cells, we applied the histone deacetylatylase inhibitor trichostatin A (TSA). TSA treatment of CIITA-deficient Jurkat T cells did not result in induction of CIITA expression driven by CIITA-PIII (Fig. 3C). Interestingly, even in the absence of functional CIITA, expression of HLA-DRA can be induced following histone deactylation upon TSA treatment (Fig. 3C). It reveals that for transcriptional activation of the HLA-DRA gene alteration of the local chromatin environment is sufficient to allow base line levels of HLA-DRA transcription in the absence of CIITA.

Therefore, the defect in CIITA expression in leukemic T cells is primarily due to DNA hypermethylation of CIITA-PIII. In this way expression of CIITA is prohibited and allows the expression of other genes that fulfil essential T cell functions. Furthermore, because T cell leukemia's are derived from normal T cells at various stages of differentiation it could be argued that methylation of CIITA promoters may play a role in these T cell selection processes and is not due to the malignant transformation of these T cells.

5. MHC class II signaling in CIITA transfected Jurkat T cells induces cell death

Apoptosis, or programmed cell death, can be triggered by a variety of stimuli, including cytotoxic T lymphocyte (CTL)-mediated killing via either the CD95 (Fas) or granzyme B/perforin-mediated pathway but also by ionising radiation and many cytostatic drugs [35]. These pathways involve activation of various caspases. In human B-lymphocytes also ligation of HLA-DR molecules, but not HLA-DP or -DQ, induces cell death [36–39], which is mediated by a caspase-independent cell death pathway [40].

To investigate whether CIITA-induced cell surface expression of HLA-DR on otherwise MHC class II-deficient leukemic T cell lines could also be used as a target to induce cell death, we introduced CIITA into Jurkat T cells. Following antibiotic selection Jurkat-MHC-II$^+$ cells were established. Taking advantage of the cell death inducing anti-HLA-DR antibody L243 [41] ligation of HLA-DR on Jurkat-MHC-II$^+$ cells resulted in induction of cell death as determined by annexin V-FITC and propidium iodide staining (Fig. 4). Similar results were obtained for HLA-DR$^+$ T cell lymphoma cell lines and B cell lines (Holling et al., unpublished observations) [37–41]. These observations reveal that similar to B cells also in T cell leukemia's and lymphomas HLA-DR molecules can mediate cell death inducing signaling.

6. Conclusions

Our investigations towards the regulation and function of MHC class II molecules in T cell malignancies have revealed a role for CpG island methylation of CIITA-PIII in T cell leukemia's, which accounts for the MHC class II-deficient phenotype of these malignancies. Furthermore, we have shown that ligation of CIITA-induced HLA-DR and resulting signaling results in induction of cell death. It can therefore be envisioned that interference in the CIITA-silencing pathways in CIITA-deficient leukemic T cells would result in induction of cell surface MHC class II expression. These MHC class II$^+$ leukemic T cells could than be used as targets for antibody therapy aimed at induction of tumor cell death mediated by HLA-DR molecules.

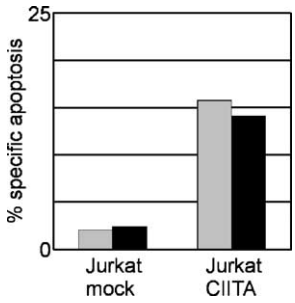

Fig. 4. CIITA-transfected Jurkat T cells (HLA-DR$^+$) were treated for 3 h (grey) or 18 h (black) with 10 μg/ml of the L243 anti-HLA-DR antibody (sc-18875, Santa Cruz Biotechnology, TEBU-BIO, Heerhugowaard, The Netherlands). Percentage of specific apoptosis was measured with annexin V-FITC/propidium iodine staining and calculated as described by Drénou et al. [40]. Mock-transfected Jurkat T cells (HLA-DR$^-$) were used a control.

Acknowledgments

PVdE is currently supported by the Dutch Cancer Society (NKB-UL: 01-2522; 03-2798) and Dr. Gisela Thier Foundation. NvdS is a Fellow of the Royal Netherlands Academy of Arts and Sciences.

References

[1] Boss JM, Jensen PE. Transcriptional regulation of the MHC class II antigen presentation pathway. Curr Opin Immunol 2003;15:105–11.
[2] Holling TM, Schooten E, Van den Elsen PJ. Function and regulation of MHC class II molecules in T-lymphocytes: of mice and men. Hum Immunol 2004;65:282–90.
[3] Al-Daccak R, Mooney N, Charron D. MHC class II signaling in antigen-presenting cells. Curr Opin Immunol 2004;16:108–13.
[4] Di Rosa F, D'Oro U, Ruggiero G, Racioppi L, Acquaviva A, Ferrone S, et al. HLA class II molecules transduce accessory signals affecting the CD3 but not the interleukin-2 activation pathway in T blasts. Hum Immunol 1993;38:251–60.
[5] Drénou B, Blancheteau V, Burgess DH, Fauchet R, Charron DJ, Mooney NA. A caspase-independent pathway of MHC class II antigen-mediated apoptosis of human B lymphocytes. J Immunol 1999;163:4115–24.
[6] Nagy ZA, Mooney NA. A novel, alternative pathway of apoptosis triggered through class II major histocompatibility complex molecules. J Mol Med 2003;81:757–65.
[7] Catlett IM, Xie P, Hostager BS, Bishop GA. Signaling through MHC class II molecules blocks CD95-induced apoptosis. J Immunol 2001;166:6019–24.

[8] Nagy ZA, Hubner B, Lohning C, Rauchenberger R, Reiffert S, Thomassen-Wolf E, et al. Fully human, HLA-DR-specific monoclonal antibodies efficiently induce programmed death of malignant lymphoid cells. Nat Med 2002;8:801–7.
[9] Altomonte M, Fonsatti E, Visintin A, Maio M. Targeted therapy of solid malignancies via HLA class II antigens: a new biotherapeutic approach? Oncogene 2003;22:6564–9.
[10] Van den Elsen PJ, Holling TM, Kuipers HF, Van der Stoep N. Transcriptional control of antigen presentation. Curr Opin Immunol 2004;16:67–75.
[11] Reith W, Mach B. The bare lymphocyte syndrome and the regulation of MHC expression. Ann Rev Immunol 2001;19:331–73.
[12] Zhu XS, Linhoff MW, Li G, Chin KC, Maity SN, Ting JP-Y. 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: 6051–61.
[13] Masternak K, Muhlethaler-Mottet A, Villard J, Zufferey M, Steimle V, Reith W. CIITA is a transcriptional coactivator that is recruited to MHC class II promoters by multiple synergistic interactions with an enhanceosome complex. Genes Dev 2000;14:1156–66.
[14] Jabrane-Ferrat N, Nekrep N, Tosi G, Esserman L, Peterlin BM. MHC class II enhanceosome: how is the class II transactivator recruited to DNA-bound activators? Int Immunol 2003;15:467–75.
[15] DeSandro A, Nagarajan UM, Boss JM. The bare lymphocyte syndrome: Molecular clues to the transcriptional regulation of major histocompatibility complex class II genes. Am J Hum Genet 1999; 65:279–86.
[16] Ting JP-Y, Trowsdale J. Genetic control of MHC class II expression. Cell 2002;109:21–33.
[17] Nekrep N, Fontes JD, Geyer M, Peterlin BM. When the lymphocyte loses its clothes. Immunity 2003;18:453–7.
[18] Gobin SJP, Van Zutphen M, Westerheide SD, Boss JM, Van den Elsen PJ. The MHC-specific enhanceosome and its role in MHC class I and β2-microglobulin gene transactivation. J Immunol 2001;167: 5175–84.
[19] 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: 8489–98.
[20] Raval A, Howcroft TK, Weissman JD, Kirshner S, Zhu XS, Yokoyama K, et al. Transcriptional coactivator, CIITA, is an acetyltransferase that bypasses a promoter requirement for TAF(II)250. Mol Cell 2001; 7:105–15.
[21] Pattenden SG, Klose R, Karaskov E, Bremner R. Interferon gamma-induced chromatin remodeling at the CIITA locus is BRG1 dependent. EMBO J 2002;21:1978–86.
[22] 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;22:5019–26.
[23] Zika E, Greer SF, Zhu XS, Ting JP. Histone deacetylase 1/mSin3A disrupts gamma interferon-induced CIITA function and major histocompatibility complex class II enhanceosome formation. Mol Cell Biol 2003;23:3091–102.
[24] Muhlethaler-Mottet A, Otten LA, Steimle V, Mach B. 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;16:2851–60.
[25] Muhlethaler-Mottet A, Di Berardino W, Otten LA, Mach B. Activation of the MHC class II transactivator CIITA by interferon-γ requires cooperative interaction between Stat1 and USF-1. Immunity 1998;8:157–66.
[26] Piskurich JF, Wang Y, Linhoff MW, White LC, Ting JP-Y. Identification of distinct regions of 5′ flanking DNA that mediate constitutive, IFNβ, STAT1, and TGF-β-regulated expression of the class II transactivator gene. J Immunol 1998;160:233–40.
[27] Piskurich JF, Linhoff MW, Wang Y, Ting JP-Y. Two distinct γ 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 β. Mol Cell Biol 1999;19:431–40.
[28] Van der Stoep N, Quinten E, Van den Elsen PJ. Transcriptional regulation of the MHC class II transactivator (CIITA) promoter III: Identification of a novel regulatory region in the 5′-UTR and an important role for CREB-1 and ATF-1 in CIITA-PIII transcriptional activation in B-lymphocytes. J Immunol 2002;169:5061–71.
[29] Holling TM, Van der Stoep N, Quinten E, Van den Elsen PJ. Activated T cells accomplish MHC class II expression through T cell specific occupation of CIITA promoter III. J Immunol 2002;168:763–70.
[30] Wong AW, Ghosh N, McKinnon KP, Reed W, Piskurich JF, Wright KL, et al. Regulation and specificity of MHC2TA promoter usage in human primary T lymphocytes and cell line. J Immunol 2002; 169:3112–9.
[31] Landmann S, Muhlethaler-Mottet A, Bernasconi L, Suter T, Waldburger JM, Masternak K, et al. Maturation of dendritic cells is accompanied by rapid transcriptional silencing of class II transactivator (CIITA) expression. J Exp Med 2001;194:379–91.
[32] Ghosh N, Piskurich JF, Wright G, Hassani K, Ting JP, Wright KL. A novel element and a TEF-2-like element activate the major histocompatibility complex class II transactivator in B-lymphocytes. J Biol Chem 1999;274:32342–50.
[33] Holling TM, Schooten E, Langerak T, Van den Elsen PJ. Regulation of MHC class II expression in human T cell malignancies. Blood 2004;103:1438–44.
[34] Saufuddin M, Roebuck KA, Chang Ch, Ting Y-P, Spear GT. Cutting edge: activation of HIV-1 transcription by the MHC class II transactivator. J Immunol 2000;164:3941–5.
[35] Rathmell JC, Thompson CB. The central effectors of cell death in the immune system. Ann Rev Immunol 1999;17:781–828.
[36] Kabelitz D, Janssen O. Growth inhibition of Epstein-Barr virus-transformed B cells by anti-HLA-DR antibody L243: possible relationship to L243-induced down-regulation of CD23 antigen expression. Cell Immunol 1989;120:21–30.
[37] Newell MK, VanderWall J, Beard KS, Freed JH. Ligation of major histocompatibility complex class II molecules mediates apoptotic cell death in resting B lymphocytes. Proc Natl Acad Sci USA 1993;90:10459–63.
[38] Vaickus L, Jones VE, Morton CL, Whitford K, Bacon RN. Antiproliferative mechanism of anti-class II monoclonal antibodies. Cell Immunol 1989;119:445–58.
[39] Truman JP, Ericson ML, Choqueux-Seebold CJ, Charron DJ, Mooney NA. Lymphocyte programmed cell death is mediated via HLA class II DR. Int Immunol 1994;6:887–96.
[40] Drénou B, Blancheteau V, Burgess DH, Fauchet R, Charron DJ, Mooney NA. A caspase-independent pathway of MHC class II antigen-mediated apoptosis of human B lymphocytes. J Immunol 1999;163:4115–24.
[41] Thibeault A, Zekki H, Mourad W, Charron D, Al-Daccak R. Triggering HLA-DR molecules on peripheral monocytes induces their death. Cell Immunol 1999;192:79–85.