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Expression of the MHC Class II Transactivator (CIITA) type IV promoter in B lymphocytes and regulation by IFN- γ

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Abstract

The MHC class II transactivator (CIITA), the master regulator of MHC class II (MHC II) expression, is a co-activator that controls MHC II transcription. Human B lymphocytes express MHC II constitutively due to persistent activity of CIITA promoter III (pIII), one of the four potential promoters (pI–pIV) of this gene. Although increases in MHC II expression in B cells in response to cytokines have been observed and induction of MHC II and CIITA by IFN- γ has been studied in a number of different cell types, the specific effects of IFN- γ on CIITA expression in B cells have not been studied. To investigate the regulation of CIITA expression by IFN- γ in B cells, RT-PCR, in vivo and in vitro protein/DNA binding studies, and functional promoter analyses were performed. Both MHC II and CIITA type IV-specific RNAs increased in human B lymphocytes in response to IFN- γ treatment. CIITA promoter analysis confirmed that pIV is IFN- γ inducible in B cells and that the GAS and IRF-E sites are necessary for full induction. DNA binding of IRF-1 and IRF-2, members of the IFN regulatory factor family, was up-regulated in B cells in response to IFN- γ and increased the activity of CIITA pIV. In vivo genomic footprint analysis demonstrated proteins binding at the GAS, IRF-E and E box sites of CIITA pIV. Although CIITA pIII is considered to be the hematopoietic-specific promoter of CIITA, these findings demonstrate that pIV is active in B lymphocytes and potentially contributes to the expression of CIITA and MHC II in these cells.

Keywords

CIITA; B lymphocytes; IFN- γ ; IRF-1; IRF-2

1. Introduction

MHC class II (MHC II) gene products are surface glycoproteins that present peptides to CD4 + T lymphocytes, and therefore play a key role in the development of both humoral and cellular effector immune responses (Cresswell, 1994). Expression of MHC II is tissue specific and regulated both developmentally and by cytokine-controlled mechanisms. MHC II expression depends on the presence of a conserved sequence motif called the class II enhancer or SXY module in the promoters of MHC II and accessory genes (Glimcher and Kara, 1992; van den Elsen et al., 2004). Although RFX, NFY, and CREB protein complexes cooperatively bind this enhancer, transcription is not activated in the absence of the MHC II transactivator (CIITA) (Masternak et al., 2000; Reith and Mach, 2001). CIITA is a non-DNA binding factor recruited to the class II enhancer complex via multiple protein-protein interactions that stabilize the complex and initiate the biological activities that result in transcription (DeSandro et al.,

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2000;Harton and Ting, 2000;Zhu et al., 2000;Spilianakis et al., 2003). Both constitutive and IFN- γ -inducible expression of MHC II are directly controlled by CIITA (Steimle et al., 1993;Steimle et al. 1994;Chang et al., 1994;Chin et al., 1994). As such, CIITA has been called the master regulator of MHC II transcription and serves as a critical master switch for MHC II antigen presentation and immune responses.

Regulated primarily at the level of transcription, CIITA expression is controlled in a complex and cell-type specific manner (Ting and Trowsdale, 2002;LeibundGut-Landmann et al., 2004). *MHC2TA*, the human CIITA gene, is controlled by at least three of four distinct promoters (pI-pIV) (Muhlethaler-Mottet et al., 1997). Of these four, the function of pII has not been pursued extensively since it is not conserved across species and transcripts originating from this promoter are rare. Each CIITA promoter unit transcribes a unique first exon so that transcripts originating from each promoter are unique, a feature that is useful in the determination of the expression patterns of these promoters.

Constitutive expression of CIITA is restricted to antigen-presenting cells with CIITA types I and III expressed constitutively in dendritic cells and, to a lesser degree, in macrophages. In addition, CIITA type III is constitutively expressed in mature B lymphocytes (Muhlethaler-Mottet et al., 1997;Landmann et al., 2001;Pai et al., 2002). Of these two promoters, CIITA pIII has been studied more extensively while little is known about CIITA pI regulation. Constitutive CIITA pIII activity in B cells as well as the activity of this promoter in activated T cells depends on seven sequence elements located upstream of pIII, which include 2 E-box motifs, the activation response elements (ARE)-1 and 2, and Sites A, B and C (Piskurich et al., 1998;Ghosh et al., 1999;Holling et al., 2002;Wong et al., 2002;van der Stoep et al., 2002;van der Stoep et al., 2004). The body of experimental evidence for CIITA pIII has lead to the current notion that it is the hematopoietic-specific promoter of CIITA (van den Elsen et al., 2004).

Besides constitutive expression, CIITA is expressed inducibly, largely in response to IFN- γ (Muhlethaler-Mottet et al., 1997;Muhlethaler-Mottet et al., 1998;Piskurich et al., 1998;Dong et al., 1999;Piskurich et al., 1999). Although CIITA pIV is considered to be the primary promoter of CIITA induced by IFN- γ in extrahematopoietic cells (Piskurich et al., 1999;Soos et al., 2001;Waldburger et al., 2001), induction of CIITA pIII by IFN- γ has been described for a number of different cell types (Nikcevic et al., 1999;Piskurich et al., 1999;Soos et al., 2001;Pai et al., 2002;van der Stoep et al., 2002;Nagabhushanam et al., 2003). A STAT1-dependent, IRF-1-independent mechanism has been described for the induction of CIITA pIII by IFN- γ (Piskurich et al., 1999). In contrast, induction of CIITA pIV occurs by a mechanism that depends on the binding of STAT1 to an IFN- γ activation factor DNA-binding sequence (GAS), the binding of IRF-1 to an IFN regulatory factor-element (IRF-E), and the binding of USF-1 to an interposed E box site (Muhlethaler-Mottet et al., 1998;Piskurich et al., 1999;Morris et al., 2002). Recent reports also demonstrate that CIITA pIV is activated by IRF-2, another member of the IFN regulatory factor family of transcriptional regulators, and indicate that IRF-2 may cooperatively enhance the induction of this promoter by IRF-1 (Xi et al., 1999;Xi and Blanck, 2003).

Increases in MHC II expression in response to cytokines have been widely reported in B cells and increases at the level of MHC II transcription in this cell type have been shown (Rousset et al., 1988;Walter et al., 2000;Pai et al., 2002). However, the induction of CIITA expression in these cells has not been well studied, especially in response to IFN- γ . The purpose of this study is to investigate the potential in B lymphocytes for the inducibility of CIITA and MHC II by IFN- γ at the level of transcription. This is biologically important since MHC II expression is central to the antigen-presenting function of B cells and these studies may provide insight into mechanisms useful for increasing the ability of the host's immune system to recognize B-cell neoplasms like multiple myeloma. We demonstrate in this report for the first time CIITA

pIV is functionally active and that IFN- γ treatment increases the activity of this promoter in B lymphocytes. We further show that the mechanism responsible for this induction includes features common to those described in non-hematopoietic cell types where CIITA expression is inducible.

2. Materials and methods

2.1. Culture and stimulation of B cell lines

Raji and BJAB human Burkitt' lymphoma cells were grown under standard conditions in RPMI 1640 with 10% fetal calf serum, 2mM L-glutamine, and 100 U/ml penicillin and streptomycin. Cells were treated with recombinant human IFN- γ (500 U/ml) for 24 h.

2.2. Isolation and stimulation of peripheral B cells

CD19+ B cells were isolated from healthy normal blood donors by negative selection using the B Cell Isolation Kit II and the AutoMACS system (Mitenyi Biotech). The cells were grown for 4 days on gamma-irradiated CD40L expressing L cells in the presence of IL-2 (20 U/ml), IL-4 (50 ng/ml), IL-10 (50 ng/ml), and IL-12 (2 ng/ml) (van Kooten et al., 1994; Tarte et al., 2002). Purity of the B cells was confirmed by flow cytometry for CD20 and CD19. Purified cells were stimulated with recombinant human IFN- γ (250 U/ml) for 24 h.

2.3. Constructs

The pIII, pIV, pmIV(GAS) and pmIV(IRF-E) CIITA promoter reporter constructs have been described previously (Piskurich et al., 1998; Piskurich et al., 1999). These constructs were formerly called pIIICIITA.Luc, pIVCIITA.Luc, pmGAS.IVCIITA.Luc (contains the GAS site mutations, ggagtcTAAA, with mutations designated by lowercase type), and pmIRF.IVCIITA.Luc (contains the IRF-E site mutations, GAAcTagAAGG), respectively. The human IRF-1 and IRF-2 expression plasmids were a kind gift of Ying Cha Henderson (Cha and Deisseroth, 2004).

2.4. RT-PCR

Total cellular RNA was isolated using TRIzol (Invitrogen) according to the manufacturer's instructions from 5×10^6 cells, then digested with RQ1 RNase-free DNase (Promega) according to manufacturer's directions. To prepare the first-strand cDNA, RNA (1 μ g) was reverse-transcribed in a reaction volume of 20 μ l using Superscript II Reverse Transcriptase and random primers (Invitrogen). Of the cDNA, 1 μ l was amplified with AmpliTaq Gold DNA polymerase (Applied Biosystems). Conditions for the PCR were as follows: MHC II (*HLADRA*), 5'-TGTTTGGACTTTGATGGTGATGAG-3' and 5'-AATAATGATGCCACCAGACC-3', 55°C, 37 cycles; CIITA pIV, 5'-AGGGAGAGGCCACCAGCAG-3' and 5'-GAACTGGTCGCAGTTGATG-3', 60°C, 38 cycles; CIITA pIII, 5'-GCCCTGCTGGGTCCTACCTG-3' and 5'-GAACTGGTCGCAGTTGATG-3', 60°C, 38 cycles; human cyclophilin, 5'-GGTTTATGTGTCAGGGTGTTG-3' and 5'-TTCATGCCTTCTTTCACTTTG-3', 60°C, 25 cycles. PCR was performed using serial dilutions of the template to verify that amplification was in the linear range for the assays (data not shown). PCR products (50% of the total reaction volume) were resolved on 1.5% agarose gels, stained with ethidium bromide and visualized using the FP-1500 Gel Analysis System (Ultra-Lum).

2.5 Transfections and luciferase assays

Raji B cells were transiently transfected by electroporation and luciferase assays were performed as previously described (Piskurich et al., 1998) with the following modifications: 6 h after electroporation, cells were treated with human recombinant IFN- γ (500 U/ml) for 24

h and then harvested; luciferase activity was measured using a Tropix TR717 luminometer (PE Applied Biosystems); as an internal control, each transfection included 300 ng of the pRL-TK Vector (Promega) which directs the expression of Renilla luciferase. Renilla luciferase activity was measured using the Renilla Luciferase Assay System (Promega) according to the manufacturer and was used to normalize the luciferase activity for transfection efficiency.

2.6. Electrophoretic mobility shift assays (EMSAs)

Nuclear extracts were prepared from 2×10^6 Raji B cells, treated with IFN- γ (500 U/ml) for 0 or 18 h, according to the manufacturer's directions using NE-PER[®] and Cytoplasmic Extraction Reagents (Pierce). The WT CIITA pIV oligonucleotide probe was as follows: 5'-CTGCAGAAAGAAAGTGAAAGGGAAAAAGAACT-3'. Oligonucleotide probes used as cold competitors were MT CIITA pIV, 5'-CTGCAGAAAGAAActtagAAGGGAAAAAGAACT-3' (mutations shown in lowercase type), and IRF-E, 5'-CGGCCGCTTTCGATTTTCGCTTTCCCCTAAATGGCTG-3', an oligonucleotide that contains the IRF-E site of the TAP1 gene (White et al., 1996). EMSAs were performed as previously described except for the following modification: 6 μ g of extract were incubated with probe for 30 min (Piskurich et al., 1999). Antibodies were purchased from Santa Cruz Biotechnology.

2.7. In vivo genomic footprinting

The dimethyl sulfate (DMS) treatment, genomic DNA preparation, amplification of human CIITA (*MHC2TA*) pIV using ligation-mediated PCR, electrophoresis and visualization were all performed as previously described (Piskurich et al., 1999). Briefly, 1×10^7 Raji B cells were treated with IFN- γ (500 U/ml) for 0, 24 or 48 h before DMS treatment and genomic DNA isolation. Three *MHC2TA* locus-specific primers were used to amplify cleaved fragments from the upper strand of pIV. Ligation-mediated PCR was also performed on the lower strand, but no significant protections or enhancements were observed.

3. Results

3.1. MHC II and CIITA Type IV-specific RNAs increase in human B cells in response to IFN- γ treatment

Each CIITA promoter controls the transcription of a unique first exon, therefore transcripts originating from the activity of individual promoters have unique sequences at their 5' termini and can be selectively amplified by RT-PCR. Although CIITA pIV is considered to be the primary CIITA promoter induced by IFN- γ in extrahematopoietic cells, induction of CIITA pIII by IFN- γ has been described for some cell types (Nikcevic et al., 1999; Piskurich et al., 1999; Soos et al., 2001; Pai et al., 2002; van der Stoep et al., 2002; Nagabhushanam et al., 2003). Since different cell types might selectively induce these promoters, it is necessary to test the responses of both CIITA pIII and pIV to IFN- γ in human B cells. To detect changes in MHC II and CIITA type III and IV RNA levels in response to IFN- γ treatment, primers were designed that detect these transcripts by RT-PCR. As a control for cDNA loading, RT-PCR was also performed using primers for human cyclophilin. RT-PCR analysis using Raji, BJAB and primary human B cells revealed that after 24 h of IFN- γ treatment, both MHC II and CIITA type IV levels were increased (Fig. 1). In addition, low levels of basal CIITA type pIV expression were detected. High levels of MHC II and CIITA type III RNA were expressed in the untreated cells, demonstrating the constitutive CIITA pIII activity that we and others have reported for B cells (Muhlethaler-Mottet et al., 1997; Piskurich et al., 1998; Ghosh et al., 1999). However, an increase in CIITA type III RNA was not detected after treatment. These experiments indicate that besides constitutive expression, MHC II expression might be augmented by IFN- γ in B lymphocytes. They demonstrate that CIITA pIV is active and that the expression of CIITA type IV is increased by IFN- γ in these cells.

3.2. IFN- γ increases CIITA pIV activity in B cells

In order to functionally test the responses of the CIITA promoters to IFN- γ , human Raji B cells were transfected by electroporation using reporter plasmids where the luciferase gene was under the control of either CIITA pIV or pIII. The pIII promoter reporter plasmid contains a large 7-kb CIITA pIII upstream region that controls the full response of this promoter to IFN- γ (Piskurich et al., 1999). The pGL2-Basic plasmid (Promega), the base vector for the CIITA promoter reporter constructs, was used as negative control. CIITA pIV was active in these cells and this activity increased after IFN- γ treatment, while no increase in activity in response to IFN- γ was observed for CIITA pIII (Fig. 2). These data are consistent with the results of the RT-PCR analysis which indicates that CIITA pIV is IFN- γ -responsive in B cells. Since pIII activity was not increased, these results further demonstrate the specificity of this response for CIITA pIV.

3.3. The STAT1 and IRF-binding sites of CIITA pIV are necessary for full activity in B cells

Mechanisms described for the regulation of CIITA pIV by IFN- γ in non-hematopoietic cells include the binding of STAT1 to a GAS sequence element in conjunction with the binding of IRF-1 to an IRF-E sequence motif (Muhlethaler-Mottet et al., 1998;Piskurich et al., 1999;Morris et al., 2002). To determine whether the activity of pIV is dependent on these elements in B cells, a promoter reporter analysis was performed to compare the activity of the unmutated CIITA pIV construct with CIITA pIV constructs with mutated GAS or IRF-E sites. While a decrease in IFN- γ responsive activity was seen for both plasmids, the decrease was more pronounced for the IRF-E mutation where basal activity was also decreased (Fig. 3). The results demonstrate for the first time that CIITA pIV is induced in B cells by a mechanism that is dependent on the GAS and IRF-E sites.

3.4. IRF-1 and IRF-2 activate CIITA pIV activity in B cells

To further examine the ability of IRF-1 to activate CIITA type IV in B cells, the effect of ectopic expression of IRF-1 on CIITA pIV activity was examined. Cotransfections were performed in Raji cells with either the unmutated CIITA pIV construct or constructs with mutated GAS or IRF-E sites and a plasmid that constitutively expresses IRF-1. Three to four fold induction of activity was observed for the unmutated CIITA pIV and for the CIITA pIV construct with the mutated GAS site (Fig. 4). In contrast, neither the CIITA pIV construct with the mutated IRF-E site nor the negative control reporter plasmid was induced. These results support the finding that the IRF-E site is necessary for induction. Additionally, they demonstrate the ability of IRF-1 to activate CIITA pIV activity in B lymphocytes.

Although IRF-2, another member of the IRF family of transcriptional regulators was initially characterized as a transcriptional repressor (Harada et al., 1989), positive regulation by IRF-2 has also been described (Vaughan et al., 1995;Luo and Skalnik, 1996;Jesse et al., 1998;Oshima et al., 2004). Recently, the ability of IRF-2 to positively regulate CIITA pIV in non-hematopoietic cells has been demonstrated (Xi et al., 1999;Xi and Blanck, 2003). These recent reports indicate that the CIITA pIV IRF-E is capable of co-occupation by IRF-1 and IRF-2. Additionally, they demonstrate facilitation of the binding of IRF-1 to this site by IRF-2 and further show that IRF-2 alone can activate CIITA pIV (Xi and Blanck, 2003). B cells constitutively express certain IRF family members, in particular IRF-4, which potentially bind the CIITA pIV IRF-E and interfere with induction of this promoter by IRF-2. To determine if IRF-2 can influence the activity of CIITA pIV in B cells, we examined the effect of the ectopic expression of IRF-2 on pIV activity. Cotransfections were performed in Raji cells with the unmutated CIITA pIV promoter reporter construct and with a plasmid that constitutively expresses IRF-2. In B cells over-expressing IRF-2 an induction of CIITA pIV activity was observed similar to the effect that was seen for B cells over-expressing IRF-1 (Fig. 5). These

experiments demonstrate for the first time that IRF-2 acts as an activator of CIITA pIV in B cells.

3.5. Genomic footprint analysis detects protein-DNA interactions at the GAS, IRF-E and E box sites of CIITA pIV in B cells

The IFN- γ -responsive region of CIITA pIV consists of a relatively small (approximately 100 bp) region of DNA located upstream of the pIV transcriptional start site (Muhlethaler-Mottet et al., 1997). In vivo genomic footprinting is the method of choice to identify physiologically relevant sequences in such a small region of the DNA. DMS-treated genomic DNA was obtained from Raji B cells that either left uninduced, or were induced with IFN- γ for 24 or 48 h. Significant protections indicating protein-DNA contacts were observed in B cells at the GAS, IRF-E and E box sites (Fig. 6). Treatment with IFN- γ resulted in a significant change in the protein-DNA contacts at the GAS site, with the central guanine residue of this site becoming protected after treatment (see asterisk). In contrast, similar protein-DNA contacts were observed at the IRF-E site before and after induction. These results are consistent with a change in protein binding for CIITA pIV in response to IFN- γ in B cells and therefore they lend further support to the findings that IFN- γ increases CIITA pIV activity in this cell type.

3.6. IRF-1 and IRF-2 bind to the CIITA pIV IRF-E in B cells

To determine whether IRF-1 and/or IRF-2 bind to the CIITA pIV IRF-E site in B cells in response to IFN- γ , in vitro electrophoretic mobility shift assays (EMSAs) were performed. One specific complex formed on this site in response to IFN- γ treatment (Fig. 7A, compare lanes 1 and 2). Specific cold competitors consisting of the CIITA pIV wild type IRF-E (lane 3) or the IRF-E site from the TAP1 gene promoter (lane 5) eliminated the formation of this complex, while competition with an oligonucleotide with a mutated IRF-E site did not (lane 4). Additional bands located above this complex changed in appearance even in competitions with the mutated CIITA pIV (compare lanes 3 and 5 with lane 4) and therefore are not specific for the CIITA pIV IRF-E site. The preincubation of nuclear extracts from IFN- γ -treated cells with anti-IRF-1 antibody resulted in a supershifted band (Fig. 7B, lane 5), while preincubation with normal serum did not (lane 4). Preincubation of extracts from treated cells with an antibody to IRF-2 reproducibly resulted in a partially supershifted band (lane 6). Taken together, these experiments demonstrate that binding of IRF-1 to the CIITA pIV IRF-E site in B cells is induced following stimulation by IFN- γ . These results also provide additional support for the functional promoter analyses, which demonstrated that the IRF-E site is necessary for up-regulation of CIITA pIV activity by IFN- γ . These findings are also consistent with the ability of IRF-2 to bind and activate CIITA pIV in B cells.

4. Discussion

MHC II molecules present peptides to CD4⁺ T lymphocytes, therefore their expression plays a key role in the development of humoral immune responses and also in the effector functions of cellular immunity. Although CIITA-independent pathways for MHC II expression have been described for endothelial cells and for cells at immune-privileged sites including the eye and brain (Collinge et al., 1998; Kuchty et al., 2003; Arancibia-Carcamo et al., 2004), CIITA expression is the major factor that controls the expression of the MHC II molecules that function to present peptide antigens to T cells during the process of B lymphocyte activation. CIITA expression is regulated primarily by transcription and in a complex cell-type specific manner that utilizes multiple promoter regions (Ting and Trowsdale, 2002; LeibundGut-Landmann et al., 2004). Because of this complexity, to understand the regulation of MHC II expression in B cells it is necessary to study the mechanisms of CIITA transcription that relate specifically to B cells. The principal CIITA promoter target for the study of CIITA expression in this cell type has been CIITA pIII due to its high constitutive level of activity in these cells. Since the

primary promoter responsible for CIITA expression in activated human T cells is also CIITA pIII (Wong et al., 2002), a major focus for the study of CIITA expression in hematopoietic cells has been CIITA pIII.

Yet even the original report which assayed the expression patterns of the multiple promoters of CIITA using RNase protection assays reveals constitutive activity of CIITA pIV in B cells (Muhlethaler-Mottet et al., 1997). We now provide several lines of evidence demonstrating CIITA pIV activity in B lymphocytes. Constitutive CIITA type IV-specific expression is seen in B cells by both semi-quantitative RT-PCR and promoter reporter analysis. In addition, in vivo footprinting detects protein-DNA interactions in B cells at CIITA pIV prior to induction by IFN- γ . These interactions seen in the absence of IFN- γ treatment include the CIITA pIV E box, a binding site for the ubiquitously expressed factor USF-1, and IRF-E, a binding site for members of the IRF family of transcriptional regulatory factors. It is possible that these sites play a role in the basal transcription of this promoter in B cells. Some members of the IRF family, including IRF-4 and IRF-8/ICSBP, are expressed in uninduced B lymphocytes and recent studies show that IRF-4 activates CIITA pIII (Nelson et al., 1993; Eisenbeis et al., 1995; van der Stoep et al., 2004). It is therefore possible that binding of a constitutively expressed IRF to CIITA pIV contributes to this basal activity. Collectively, our data demonstrate that besides CIITA pIII, CIITA pIV is expressed in B cells and may contribute to the overall levels of CIITA transcription in these cells.

Studies of CIITA pIV have focused on its mechanism of induction in non-hematopoietic cells (Muhlethaler-Mottet et al., 1998; Piskurich et al., 1999; Xi et al., 1999; Morris et al., 2002). In the cell types that have been examined thus far, induction of pIV by IFN- γ relies on the binding of STAT1, IRF-1 and USF-1 to GAS, IRF-E and E box sequence motifs respectively. Recent reports have also indicated that CIITA pIV activity is up-regulated by IRF-2 expression (Xi and Blanck, 2003). In agreement with these findings, we now show that induction of pIV in B cells is dependent on the binding sites for STAT1 and IRF-1 and further demonstrate that ectopic expression of either IRF-1 or IRF-2 up-regulates its activity in B lymphocytes. In the promoter reporter analysis, a decrease in the IFN- γ response was seen when either the GAS or IRF-E sites were mutated, with the decrease more pronounced for the IRF-E mutation (Fig. 3). This result is consistent with our experiments in human fibroblasts (unpublished observations) and also with the results of another group studying analogous CIITA pIV mutations in astrocytes (Dong et al., 1999); therefore, it is not specific for B lymphocytes. Ectopic over-expression of IRF-1 alone without IFN- γ treatment was sufficient to activate CIITA pIV activity, a finding similar to those for experiments performed in non-hematopoietic cells including mouse P19 carcinoma cells and embryonic fibroblasts that lack IRF-1 (Piskurich et al., 1999; Xi et al., 1999; Xi and Blanck, 2003). Thus, we demonstrate for the first time that CIITA pIV can be up-regulated by IFN- γ in B cells by mechanisms similar to those described previously for non-hematopoietic cells. Besides these similarities, differences are also noted for the induction of CIITA by IFN- γ in B cells. We and others have demonstrated up-regulation of both CIITA pIII and pIV by IFN- γ for several cell types (Nikcevich et al., 1999; Piskurich et al., 1999; Soos et al., 2001; Pai et al., 2002; van der Stoep et al., 2002; Nagabhushanam et al., 2003). However, the activity of pIII was not up-regulated by IFN- γ in either the RT-PCR or promoter reporter analysis in B lymphocytes. Therefore, unlike cell types where both pIII and pIV are induced, it is likely that CIITA pIII activity is not significantly up-regulated by IFN- γ in B lymphocytes.

The expression of CIITA pIV in B lymphocytes might be important to the development of future therapies for multiple myeloma, a B cell neoplasm characterized by clonal expansion of malignant plasma cells for which there is no cure. Plasma cells express PRDI-BF1, called BLIMP-1 in mice, which suppresses CIITA and MHC II expression by repressing the activity of CIITA pIII (Piskurich et al., 2000; Ghosh et al., 2001). Deficiencies in CIITA expression

may contribute to the ability of multiple myeloma cells to escape immune recognition. It is possible that CIITA and MHC II expression can be activated in myeloma cells by activation of CIITA pIV, which could prove important in enhancing anti-tumor immune responses. DNA methylation of CIITA pIII and pIV limits the expression of CIITA and MHC II in human developmental tumors and many hematopoietic tumors, including myeloid and T cell malignancies (van den Elsen et al., 2003; Holling et al., 2004; Morimoto et al., 2004). However, it is noteworthy that among hematopoietic tumor cells, myeloma cells are an exception in that CIITA pIV is unmethylated in most of the cell lines that have been examined. A recent report studying HLA-DR induction in hematopoietic tumor cells demonstrated basal expression for CIITA pIV in nearly all of the B cell acute lymphocytic leukemia and myeloma cell lines that were examined (Morimoto et al., 2004). While only some of the cell lines show increases in CIITA type IV RNA in response to IFN- γ , the assay was performed after 48 h of IFN- γ treatment. We are presently examining CIITA type IV RNA expression in human myeloma cells and have observed increases in response to IFN- γ at 18–24 h time points when induction of CIITA pIV by IFN- γ is maximal (unpublished results) (Chang et al., 1994). Since several reports indicate that myeloma cells may be activated by IFN- γ to increase MHC expression and act as antigen presenting cells (Yi et al., 1997; Beatty and Paterson, 2001), defining this activity could prove to be an important area of investigation for increasing the immunogenicity of myeloma cells as therapy for this hematological malignancy.

In summary, we have investigated the expression and regulation of the CIITA type IV promoter in human B cells by IFN- γ . Our results indicate that this promoter is active and that its activity increases in response to IFN- γ . As such CIITA pIV may contribute to the expression of CIITA in B lymphocytes and may prove to be a useful target for increasing the expression of CIITA in leukemic B cells and myeloma cells to activate the ability of these cells to act as antigen presenting cells.

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Fig 1.

Increased levels of MHC class II and promoter IV-specific CIITA expression are observed in B cells in response to IFN- γ treatment. Equal amounts of RNA from Raji, BJAB and primary B cells, treated for 0 or 24 h with recombinant human IFN- γ , were subjected to RT-PCR using primers specific for the MHC II (*HLADRA*), CIITA pIII and pIV RNAs. RT-PCR using primers for human cyclophilin was performed as an internal standard for cDNA loading. This experiment has been repeated twice with similar results.

Fig 2.

A specific increase in human CIITA (*MHC2TA*) pIV activity is observed in B cells in response to IFN- γ . Transient transfections of Raji B cells were performed by electroporation using 10 μ g of luciferase reporter constructs under the control of CIITA pIV or CIITA pIII or using 10 μ g of -Control, pGL2-Basic. After a 6 h recovery period, half of the cultures were treated with 500 U/ml of recombinant human IFN- γ for 24 h before cultures were harvested. Luciferase activity was measured and normalized to Renilla luciferase activity as described above. Bars show the s.e.m., $n = 3$. At least three independent experiments have been performed with similar results.

Fig 3.

The response of CIITA pIV to IFN- γ in B cells requires the STAT1 and IRF-1-binding sites. Transient transfections of Raji B cells were performed by electroporation using 10 μ g of a luciferase reporter construct containing unmutated CIITA pIV (pIV), pIV with a mutated STAT1-binding site (GAS) [pmIV(GAS)], pIV with a mutated IRF-1-binding site (IRF-E) [pmIV(IRF-E)] or 10 μ g of -Control, pGL2-Basic. For reference, the sequence of unmutated CIITA pIV and locations of the GAS and IRF-E are shown in Fig. 6. After a 6 h recovery period, half of the cultures were treated with 500 U/ml of recombinant human IFN- γ for 24 h before harvesting and measurement of luciferase activity. Bars show the s.e.m., n = 4. At least three independent experiments have been performed with similar results.

Fig 4.

IRF-1 induces the activity of CIITA pIV in B cells. Transient cotransfections of Raji B cells were performed by electroporation using 10 μ g of IRF-1 expression plasmid or the empty expression plasmid vector, and 10 μ g of a luciferase reporter construct containing either unmutated CIITA pIV (pIV), pIV with a mutated STAT1-binding site (GAS) [pmIV(GAS)], pIV with a mutated IRF-1-binding site (IRF-E) [pmIV(IRF-E)] or 10 μ g of the -Control, pGL2-Basic. After 24 h, cells were harvested and luciferase activity was measured. Bars show the s.e.m., $n = 3$. At least three independent experiments have been performed with similar results.

Fig 5.

IRF-2 induces the activity of CIITA pIV in B cells. Transient cotransfections of Raji B cells were performed by electroporation using 10 μ g of an IRF-1 expression plasmid, an IRF-2 expression plasmid or the empty expression plasmid vector, and either 10 μ g of -Control, pGL2-Basic or the CIITA pIV luciferase reporter construct. After 24 h, cells were harvested and luciferase activity was measured. Bars show the s.e.m., n = 3. At least three independent experiments have been performed with similar results.

Fig 6.

The in vivo footprint of CIITA pIV in B cells reveals protein-DNA contacts within the GAS, IRF-E and E box sites. The sequence of the promoter region is shown with boxes indicating the relevant cis-elements. Genomic footprints of the upper strand are shown in the lower panel. Lane 1 shows genomic DNA that was methylated in vitro to reveal the complete guanine ladder. Lanes 2 through 4 show the results of IFN- γ treatment using Raji B cells treated with DMS in culture. Open arrows indicate bases protected from modification. While a number of bases in the E box and IRF-E sites are protected both before and after treatment (indicated by arrows), one central base in the GAS site becomes protected after IFN- γ treatment (see asterisk).

Fig 7.

Binding of IRF-1 and IRF-2 to CIITA pIV is induced in B cells in response to IFN- γ . (A) EMSA analysis demonstrates that a specific protein complex is induced by IFN- γ treatment (lane 1 versus lane 2) (arrow). Nuclear extracts were from Raji B cells treated with IFN- γ (500 U/ml) for 0 (UNT) or 18 h (IFN γ). The probe spans the CIITA pIV IRF-E. Cold oligonucleotide competitors, used at 50-fold molar excess (50X), are designated in the top row. Abbreviations: WT, homologous CIITA pIV competitor; MT, CIITA pIV competitor with mutated IRF-E site; IRF-E, cold competitor with the IRF-E site of the TAP1 gene (White et al., 1996); NUC. EX., nuclear extract. (B) Incubation with anti-IRF-1 or anti-IRF-2 induces a supershifted or partially supershifted complex, respectively (see asterisk). Antibodies are indicated at the top. Abbreviations: AB, antibody; NS, normal serum.