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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., 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, 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.

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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, 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, 1998; Piskurich et al., 1998, 1999; Dong et al., 1999). Although CIITA pIV is considered to be the primary promoter of CIITA induced by IFN-γ in extra-hematopoietic 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 (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). 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-hematopoetic 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's lymphoma cells were grown under standard conditions in RPMI 1640 with 10% fetal calf serum, 2 mM 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, 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, GAActTagAAGG), 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