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MHC class II transactivator (CIITA) expression is upregulated in multiple myeloma cells by IFN-γ

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Abstract

The MHC class II transactivator (CIITA) acts in the cell nucleus as the master regulator of MHC class II (MHC II) gene expression. It is important to study CIITA regulation in multiple myeloma since MHC expression is central to ability of myeloma cells to present antigen and to the ability of the immune system to recognize and destroy this malignancy. Regulation of CIITA by IFN-γ in B lymphocytes occurs through the CIITA type IV promoter (pIV), one of the four potential promoters (pI–pIV) of this gene. To investigate regulation of CIITA by IFN-γ in multiple myeloma cells, first the ability of these cells to respond to IFN-γ was examined. RT-PCR analyses show that IFN-γR1, the IFN-γ-binding chain of the IFN-γ receptor, is expressed in myeloma cells and IRF-1 expression increases in response to IFN-γ treatment. Western blotting demonstrates that STAT1 is activated by phosphorylation in response to IFN-γ. RT-PCR and functional promoter analyses show that IFN-γ upregulates the activity of CIITA pIV, as does ectopic expression of IRF-1 or IRF-2. In vivo protein/DNA binding studies demonstrate protein binding at the GAS, E box and IRF-E sites. In vitro studies confirm the binding of IRF-1 and IRF-2 to CIITA pIV. Although multiple myeloma cells express PRDI-BF1/Blimp-1, a factor that represses both the CIITA type III and IV promoters, they retain the capability to upregulate CIITA pIV and MHC II expression in response to IFN-γ treatment. These findings are the first to demonstrate that although PRDI-BF1/Blimp-1 diminishes the constitutive ability of these cells to present antigen by limiting CIITA and MHC II expression, it is possible to enhance this expression through the use of cytokines, like IFN-γ.

Keywords: Myeloma; CIITA; IFN-γ; IFN-γR1; STAT1; IRF-1; IRF-2; PRDI-BF1; Blimp-1

1. Introduction

Multiple myeloma, the most common primary malignancy of bone in older adults, is characterized by the clonal expansion of malignant plasma cells. These cells produce monoclonal immunoglobulins that carry unique antigenic determinants and act as tumor-specific antigens for recognition and destruction of these cells by the immune system (Wen et al., 2001; Galea et al., 2002). Immune responses are tightly regulated by CD4+ T helper (TH) lymphocytes (Cresswell, 1994). Activation of TH cells depends on the recognition of antigens presented by MHC class II (MHC II). MHC II-recognizing TH cells secrete cytokines that initiate and sustain CD8+ cytotoxic lymphocyte (CTL) anti-tumor responses. Therefore, MHC II expression is central to the recognition and destruction of tumor cells by CTLs. Since myeloma cells generally express low levels of the MHC class II transactivator (CIITA), which controls the expression of MHC II, they can escape immune inspection due to their decreased ability to present antigen (Ghosh et al., 2001). Loss of MHC II expression in B cell lymphomas is considered as tumorigenic and an immune escape mechanism (Fuji and Iribe, 1986; Amiot et al., 1998). Consistent with this idea, forced CIITA expression has been shown to restore MHC II expression and decrease the tumorigenicity of some tumor cell types (Shi et al., 2006).

CIITA is a non-DNA binding protein factor that is recruited to the enhancer complex of MHC II genes (Harton and Ting,

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2000). This factor is the predominant regulator of MHC II expression and CIITA expression acts as a crucial master switch for immune responses. Expression of CIITA is regulated in a complex cell-type specific manner, predominantly at the level of transcription (Ting and Trowsdale, 2002; Leibundgut-Landmann et al., 2004). The human CIITA gene, *MHC2TA*, has four distinct promoters designated as promoters I–IV and it is controlled by at least three of these (Muhlethaler-Mottet et al., 1997). While the CIITA type I promoter (pI) is mainly expressed in dendritic cells and macrophages, the type III promoter (pIII) is constitutively expressed in B lymphocytes, which constitutively express MHC II (Muhlethaler-Mottet et al., 1997; Landmann et al., 2001; Pai et al., 2002). Recently, we have also described CIITA type IV promoter (pIV) expression that is regulated by IFN-γ in B lymphocytes (Piskurich et al., 2006). Similar to the mechanisms described for other cell types where CIITA pIV expression is IFN-γ-inducible, the mechanism for the regulation of CIITA pIV by IFN-γ in B cells depends on the binding of signal transducer and activator of transcription (STAT)1 to an IFN-γ activation factor DNA-binding sequence (GAS), IFN regulatory factor (IRF)-1 and IRF-2 to an IFN regulatory factor-element (IRF-E), and the ubiquitously expressed factor, USF-1, to an interposed E box site (Muhlethaler-Mottet et al., 1998; Piskurich et al., 1999; Xi et al., 1999; Morris et al., 2002).

Positive regulatory domain I-binding factor 1 (PRDI-BF1), called B lymphocyte-induced maturation protein-1 (Blimp-1) in mice, is a transcriptional repressor that plays a pre-eminent role in the terminal differentiation of B lymphocytes into plasma cells (Turner et al., 1994; Lin et al., 1997; Shapiro-Shelef and Calame, 2005). This genetic program of B cell differentiation, which is largely intact in myeloma cells, includes the loss of constitutive MHC II expression via extinction of CIITA pIII activity by PRDI-BF1/Blimp-1 (Piskurich et al., 2000; Ghosh et al., 2001). Since myeloma cells express PRDI-BF1/Blimp-1, they also generally express low constitutive levels of CIITA and MHC II (Nagy et al., 2002). We and others have recently described the IFN-γ-inducible CIITA promoter, pIV, as a new target for repression by PRDI-BF1/Blimp-1 (Tooze et al., 2006; Chen et al., 2007). Although they express PRDI-BF1/Blimp-1, increases in MHC II expression in response to IFN-γ have been described both in vivo and in vitro for myeloma cells (Yi et al., 1997; Beatty and Paterson, 2001). While extinction of constitutive MHC II and CIITA pIII expression has been studied in myeloma cells (Ghosh et al., 2001), induction of CIITA by IFN-γ in these cells remains virtually unstudied, despite several reports indicating that multiple myeloma cells can be activated by IFN-γ to increase MHC II expression and act as antigen presenting cells (Yi et al., 1997; Beatty and Paterson, 2001). Here, we demonstrate for the first time that the IFN-γ signaling pathway for gene induction is intact in myeloma cells and that IFN-γ upregulates CIITA type IV promoter activity. This is important because it describes a mechanism by which cytokines, like IFN-γ, are potentially useful in therapies aimed at enhancing the ability of the immune system to recognize myeloma cells. Knowledge of this response is important to the discovery of ways to increase the immunogenicity and decrease the tumorigenicity of these cells by increasing their expression of CIITA and MHC II.

2. Materials and methods

2.1. Culture and stimulation of myeloma and B cell lines

The human myeloma cells lines, U266 and RPMI 8266, and Raji B lymphocytes were grown according to American Type Culture Collection specifications in RPMI 1640 with 10% fetal calf serum, 2 mM L-glutamine, and 100 U/ml penicillin and streptomycin. NCI-H929 human myeloma cells were grown similarly, except 2-mercaptoethanol (50 μm) was added to the culture medium. Cells were treated with recombinant human IFN-γ (500 U/ml, R&D Systems) as indicated below.

2.2. Constructs

The pIV, pmIV(GAS) and pmIV(IRF-E) *MHC2TA* promoter reporter constructs have been described previously (Piskurich et al., 1998; Piskurich et al., 1999). These constructs were formerly called 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).

2.3. RT-PCR

Total cellular RNA was isolated using TRIzol (Invitrogen) according to the manufacturer's instructions from 5×10^6 cells, and then digested with RQ1 RNase-free DNase (Promega) according to manufacturer's directions. To prepare the first-strand cDNA, RNA (1 μg) was reverse-transcribed in a reaction volume of 20 μl using Superscript II Reverse Transcriptase and random primers (Invitrogen). Of the cDNA, 1 μl was amplified with AmpliTaq Gold DNA polymerase (Applied Biosystems). Conditions for the PCR and sizes of the amplified products were as follows: IFN-γR1, 5′-GTTAAAGCCAGGGTTGGACA-3′ and 5′-ATCGACTTCCTGCTCGTCTC-3′, 60 °C, 27 cycles, 177 bp; IRF-1, 5′-GAAGTCCAGCCGAGATGC-3′ and 5′-CGGCACAACTTCCACTG-3′, 60 °C, 24 cycles, 235 bp; IRF-2, 5′-CCACTGAGAGCGACGAGC-3′ and 5′-GTTGGAAGTGACGAAGGACG-3′, 60 °C, 25 cycles, 236 bp; CIITA pIV, 5′-AGGGAGAGGCCACCAGCAG-3′ and 5′-GAACTGGTCGCAGTTGATG-3′, 60 °C, 37 cycles, 227 bp; MHC II (HLADRA), 5′-TGTTTGACTTTGATGGTGATGAG-3′ and 5′-AATAATGATGCCCA CCAGACC-3′, 55 °C, 28 cycles, 558 bp; PRDI-BF1, 5′-TTCAAGTATGCCACCAACAG-3′ and 5′-AATGTTAGAACGGTAGAGGTC-3′, 55 °C, 25 cycles, 549 bp; PRDI-BF1β, 5′-GTGGTGGGTTAATCGGTTTG-3′ and 5′-ATAGCGCATCCAGTTGCTTT-3′, 55 °C, 30 cycles, 172 bp; GAPDH, 5′-ACCACAGTCCATGCCATCAC-3′ and 5′-TCCACCACCCTGTTGCTGTA-3′, 60 °C, 25 cycles, 452 bp. In all cases, PCR was performed as demonstrated in Fig. 1B using