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Role of PKCδ in IFN-γ-inducible CIITA gene expression☆

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Abstract

The class II transactivator (CIITA) is a key regulatory factor for MHC class II expression. Here, we demonstrate that PKCδ plays an important role in regulating IFN-γ-inducible CIITA gene expression in macrophages. Inhibition of PKCδ by either a PKCδ inhibitor or a dominant negative (DN) mutant form of PKCδ led to down-regulation of CIITA expression. The decrease in CIITA expression by PKCδ inhibition was in part due to the reduced recruitment of serine 727-phosphorylated Stat1 and histone acetyltransferases to the CIITA promoter. As a result, IFN-γ induced histone acetylation at the CIITA promoter is also compromised. However, inhibition of PKCδ did not affect IRF-1 expression or IRF-1 binding to the CIITA promoter. Therefore, we report, for the first time, that PKCδ is an essential signaling molecule to achieve the maximal expression of CIITA in response to IFN-γ in macrophages. In addition, although IRF-1 is a key transcription factor to activate the IFN-γ inducible CIITA promoter, the effect of PKCδ on CIITA expression is mediated primarily by serine phosphorylation of Stat 1.

Keywords: Monocytes/macrophages; MHC; Protein kinases; Stat1; Transcription factors; Gene regulation

1. Introduction

Proper MHC class II expression is essential to activate CD4 T cells and to mount an adaptive immune response. MHC class II genes are constitutively expressed in B cells and dendritic cells (DC) and can be induced in macrophages by IFN-γ (Steinman et al., 1999; Reith et al., 2005). Both constitutive and inducible MHC class II gene expression requires the class II transactivator (CIITA) (Reith et al., 2005; Steimle et al., 1993; Chang et al., 1996; LeibundGut-Landmann et al., 2004a,b). CIITA is not a DNA binding protein. Instead, it acts as a scaffolding protein by interacting with itself and other transcriptional factors to activate the MHC class II promoter (Masternak et al., 2000; Sisk et al., 2001; Linhoff et al., 2001). CIITA transcription is controlled by at least three distinct promoters known as pI, pIII and pIV each of which generates unique CIITA transcripts (O'Keefe et al., 2001; Muhlethaler-Mottet et al., 1997; LeibundGut-Landmann et al., 2004a,b). pI and pIII are active in DC, whereas pIII is used in B cells and plasmacytoid DC (Muhlethaler-Mottet et al., 1997; LeibundGut-Landmann et al., 2004a,b). In macrophages, however, all three promoters can be induced by IFN-γ although the major form is transcribed from pIV (O'Keefe et al., 2001; Muhlethaler-Mottet et al., 1997; LeibundGut-Landmann et al., 2004a,b). Thus, each CIITA promoter is used in a cell type-specific manner.

pIV is controlled by three major *cis*-acting elements: an IFN-γ activation sequence (GAS), an E box and an interferon regulatory factor (IRF) element, which bind the transcription factor Stat1, USF-1, and IRF-1, respectively (O'Keefe et al., 2001; Muhlethaler-Mottet et al., 1997, 1998). IFN-γ activates tyrosine kinase JAK1 and JAK2, which results in phospho-

Abbreviations: DC, dendritic cell; IRF-1, interferon regulatory factor-1; PKC, protein kinase C; HAT, histone acetyltransferases; DN, dominant negative; ChIP, chromatin immunoprecipitation; qRT-PCR, quantitative real-time PCR; HPRT, hypoxantine guanine phosphoribosyl transferase

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rylation of Stat1. Activated Stat1 dimerizes and translocates to the nucleus, where it binds to the GAS element in pIV (Shuai et al., 1994; Wen et al., 1995; Vinkemeier et al., 1996; Mowen and David, 2000; Darnell, 1997). Stat1 also controls IRF-1 expression, which in turn activates the CIITA promoter (Piskurich et al., 1999; Morris et al., 2002). Therefore, IFN-γ mediated activation of pIV requires both Stat1 and IRF-1.

PKCs, serine/threonine-specific protein kinases, are known to regulate IFN-γ inducible expression of MHC class II (Smith et al., 1992; Giroux et al., 2003; Benveniste et al., 1991; Setterblad et al., 1998; Lee et al., 1995). Microinjection of PKC protein into peritoneal macrophages induced MHC class II expression (Smith et al., 1992). This induction was blocked by PKC inhibitors such as staurosporine, calphostin C, or H7 (Smith et al., 1992). MHC class II expression induced by IFN-γ in astrocytes is also down regulated when cells were treated with the pan-PKC inhibitor H7 (Benveniste et al., 1991). In the same study, the authors also showed that PMA treatment that activates PKC signaling does not affect MHC class II expression. In promyelocytic cells, however, PMA treatment reduced MHC class II expression by destabilizing CIITA mRNA (De Lerma Barbaro et al., 2005), suggesting a cell type specific effect of PKC. MHC class II expression requires CIITA and therefore PKC-mediated modulation of MHC class II is possibly due to a change in CIITA expression. Indeed, PKCα regulates IFN-γ-inducible expression of the CIITA gene via control of transactivation activity of IRF-1 (Giroux et al., 2003). Recently, we have demonstrated that constitutive expression of CIITA in B cells depends on PKCδ, which involves CREB phosphorylation and recruitment, but not regulation of IRF-1 (Kwon et al., 2006).

PKCδ is known to participate in both type I and II IFN signaling pathways (Uddin et al., 2002; Deb et al., 2003). PKCδ activated by type I IFN associates with Stat1, and is involved in phosphorylation of Stat1 on serine 727 (Uddin et al., 2002). Furthermore, treating cells with a PKCδ inhibitor or a dominant negative mutant form of PKCδ prevents type I IFN-dependent gene transcription (Uddin et al., 2002). Similarly, PKCδ is activated by type II IFN, and phosphorylates Stat1(Ser727) in promyelocytic cells (Deb et al., 2003). Likewise, CaMKII expression is involved in serine phosphorylation of Stat1 in response to IFN-γ (Sun et al., 2005).

In this report, we demonstrate that PKCδ is essential for maximum IFN-γ inducible CIITA gene expression. PKCδ promoted the recruitment of serine phosphorylated Stat1 and histone acetyltransferases (HATs), but not IRF-1, to the CIITA promoter. As a result, histone acetylation of the CIITA promoter was enhanced by PKCδ. However, Stat1 dependent IRF-1 expression was not affected by PKCδ. The differential effect of PKCδ on CIITA and IRF-1 expression was at least partly due to a differential requirement for Stat1 serine phosphorylation. Activation of the CIITA promoter required both tyrosine and serine phosphorylation of Stat1, whereas serine phosphorylated Stat1 was not necessary to activate the IRF-1 expression. Together, PKCδ plays an important role in achieving the maximal induction of macrophage CIITA gene expression in response to IFN-γ.

2. Materials and methods

2.1. Cells and reagents

The murine macrophage cell line RAW264.7 and Stat1 deficient human fibrosarcoma cell line U3A were maintained in DMEM and 10% FBS and antibiotics. Bone marrow-derived macrophages were generated from C57BL/6 mice that were purchased from the Jackson Laboratory (Bar Harbor, ME). In brief, total BM cells depleted of RBC, T cells, B cells, and other MHC class II-positive cells were cultured 10 days in RPMI1640 supplemented with 5% FBS and 10 ng/ml murine rM-CSF.

Murine and human rIFN-γ were obtained from BD Pharmingen (San Diego, CA), and Murine rM-CSF was from R&D Systems (Minneapolis, MN). Antibodies against CIITA, PKCδ, IRF1, and HA were purchased from Santa Cruz (Santa Cruz, CA), and anti-RFX5 was obtained from Rockland, INC (Gilbertsville, PA). Antibodies used for flow cytometry, FITC-conjugated MHC class I (H-2K^b, clone AF6-88.5), and PE-conjugated MHC class II (I-A^b, clone AF6-120.1) were obtained from BD Biosciences. Antibodies against phospho-PKCδ(Thr505) and phospho-PKCδ(Ser623), phospho-Stat1(Tyr701) and phospho-Stat1(Ser727), and Stat1 were from Cell Signaling Technology (Berverly, MA). For the ChIP assay, anti-Stat1, IRF-1, CBP, and p300 antibodies were purchased from Santa Cruz, and histone H4 was obtained from Upstate Biotechnology (Lake Placid, NY). The PKC inhibitor, Ro-31-8225, Go-6976, and Rottlerin were obtained from Cal Biochem (La Jolla, CA).

2.2. Plasmid and transfections

Wild type (WT) and the dominant negative (DN) mutant form of PKCδ have been described previously (Giroux et al., 2003; Soh and Weinstein, 2003). To generate stable cell lines expressing WT or DN PKCδ, RAW264.7 cells were transfected with WT, DN PKCδ or the empty vector, and selected with G418. The 1.1 kb pIV-driven luciferase plasmid and MHC class II Eα driven luciferase reporter plasmid were described previously (Sisk et al., 2003; Yao et al., 2006). The IRF-1 promoter construct that contains the GAS and NF-κB site (0.7 kb) was kindly provided by Dr. Richard Pine (Public Health Research Institute, New York, NY) (Pine, 1997). Transient transfection to RAW264.7 cells was performed with 2 μg of the luciferase reporter (5×10^5cells) using the lipofectamine method (Invitrogen, Carlsbad, CA). After 36 h of transfection, IFN-γ (10 ng/ml) was added with or without Rottlerin for additional 10 h. In some experiments, the reporter was co-transfected with WT or DN PKCδ for 48 h followed by IFN-γ treatment for 12 h. WT, Stat1(Y701F), and Stat1(S727A) constructs were as described (Zhang et al., 2005). U3A cells were transfected with the reporter together with WT, Stat1(Y701F), Stat1(S727A) or the empty vector (EV) using the calcium phosphate method. Two days later, the cells were treated with human IFN-γ for 12 h. Cell lysates were prepared to measure the luciferase activity as described (Sisk et al., 2003). Luciferase activity was normalized with CMV promoter driven β-galactosidase