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Distinct role of IL-3 promoter and enhancer region in murine mast cells

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Abstract

Crosslinking of Fcε receptor on mast cells induces IL-3 gene expression with the concentration dependent of intracellular calcium, but its regulatory mechanism remains unclear. Here, we found that phorbol 12-myristate 13-acetate (PMA) alone did not induce IL-3 gene expression, but potentiated A23187-induced IL-3 gene expression. Interestingly, the A23187-induced IL-3 promoter activity was suppressed by PMA, but it was enhanced when IL-3 promoter contained enhancer region, a DH site. While IL-3 mRNA expression was increased by A23187 and PMA in a dose-dependent manner, the promoter activity appeared all or none in all doses of A23187 and PMA. IL-3 promoter region between −293 and −150 bp was responsible for A23187-induced gene expression and PMA- or cyclosporin A (CsA)-mediated suppression. Taken together, IL-3 gene expression was primarily regulated at the transcriptional level, which was differentially controlled by a restricted promoter and enhancer region.

Keywords: Intracellular calcium; Gene regulation; Cytokines; IL-3; Transcription; Mast cells

1. Introduction

Interleukin 3 (IL-3) affects a wide variety of hematopoietic cells. IL-3 supports the *in vitro* proliferation and differentiation of a broad range of hematopoietic progenitor cells to develop erythrocytes, granulocytes, monocytes, megakaryocytes, and mast cells (Dexter and Spooncer, 1987). In addition, it influences the growth and activation of lymphocytes (Kimoto et al., 1988), promotes the self-renewal of hematopoietic progenitor cells (Kobayashi et al., 1989), and acts upon mature leukocytes to enhance their functional activity (Rothenberg et al., 1988).

T lymphocytes in response to *in vitro* stimulation with mitogens or antigens activate to secret IL-3 and other lymphokines (Kelso and Gough, 1988). Mast cells also produce IL-3 and other lymphokines, including granulocyte-macrophage colony-stimulating factor (GM-CSF), following activation of the immunoglobulin E receptors (Kohno et al., 2005; Plaut et al., 1989).

In the mouse, IL-3 and GM-CSF genes are 14 kb apart on chromosome 11 (Lee and Young, 1989). Some *cis*-acting elements including CK-1, CK-2, and CLE0 are found in the IL-2, IL-3, IL-4, IL-5, and GM-CSF promoters (Miyatake et al., 1988, 1991; Himes et al., 1993; Masuda et al., 1993). Such similarity in promoter sequences suggests that both IL-3 and GM-CSF expression in mouse and human T cells might be co-modulated

Abbreviations: PKC, protein kinase C; CAT, chloramphenicol acetyltransferase; PMA, phorbol 12-myristate 13-acetate; GM-CSF, granulocyte-macrophage colony-stimulating factor; CsA, cyclosporin A; TNF-α, tumor necrosis factor-α; DH site, DNase I-hypersensitive site; pIL3-1003F, pSV0CAT plasmid containing murine IL-3 promoter region (−1003 to +10 bp) with forward orientation to CAT gene; pIL3-1003E, pIL3-1003F containing the second intron region (281 bp fragment) at *Bam*HI site of pSV0CAT; pEIL3-1003, pIL3-1003F plasmid containing murine DH site of GM-CSF/IL-3 locus (603 bp fragment) at *Bam*HI site of pSV0CAT

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by common regulatory machinery, although the mechanisms that regulate IL-3 gene expression are not sufficiently understood.

Regulation of IL-3 gene expression is controlled at both the transcriptional and post-transcriptional level in human T cells (Ryan et al., 1991; Dokter et al., 1993). In primary mast cells, IL-3 production follows activation of the IgE receptor by a mechanism involving a rise in the intracellular calcium concentration (Kohno et al., 2005; Plaut et al., 1989; Wodnar-Filipowicz and Moroni, 1990). In IL-3 transcripts, several AUUUA motifs, which make up the AU-rich element (ARE) in the 3′-untranslated region (3′-UTR), direct rapid deadenylation and degradation (Stoecklin et al., 1994). In mast cells stimulated with the calcium ionophore, A23187, IL-3 production results from prolonged mRNA stability (Wodnar-Filipowicz and Moroni, 1990; Stoecklin et al., 1994).

The immunosuppressive drug cyclosporin A (CsA), upon forming a complex with cyclophilin A or B, binds to and inhibits the calcium-calmodulin-dependent Ser/Thr phosphatase calcineurin that dephosphorylates nuclear factors of activated T cells (NFAT) (Aceves et al., 2004; Shaw et al., 1995). Consequently, CsA blocks translocation of NFAT into the nucleus and inhibits the expression of various genes mediated by NFAT. A DNase I-hypersensitive (DH) site, located between murine IL-3 and GM-CSF locus, has been shown to act as an enhancer in human Jurkat T cells upon activation with PMA and calcium ionophore (Osborne et al., 1995). This region contains three putative binding sites for NFAT and other sites for *trans*-acting factors that regulate the transcriptional activity of cytokine genes. In A23187/PMA-activated Jurkat T cells, CsA inhibited the enhancer function of the DH site. In addition to the effect of CsA on transcriptional regulation of the IL-3 gene, CsA destabilizes abnormally stable IL-3 transcripts in mast cell lines (Nair et al., 1994). Despite a number of studies regarding the CsA sensitivity on IL-3 gene expression (Osborne et al., 1995) and IL-3 mRNA stability (Wodnar-Filipowicz and Moroni, 1990; Nair et al., 1994), the regulatory mechanisms of murine IL-3 gene transcription by CsA are not studied in murine mast cells.

In this study, we found that murine IL-3 gene expression was regulated at the transcriptional level as well as post-transcriptional level by intracellular calcium. The transcriptional activity was controlled by IL-3 promoter region between −293 and −150 bp, which also plays an important role in response to PMA or CsA.

2. Materials and methods

2.1. Reagents

Reagents were purchased from the following sources: calcium ionophore, A23187, from Boehringer Mannheim (Mannheim, Germany); CsA and PMA from Sigma (St. Louis, MO); Ro-31-8220 from Calbiochem (La Jolla, CA); Okadaic acid and staurosporin from GIBCO BRL (Grand Island, NY); radioisotope 1-deoxydichloroacetyl-1-[^{14}C]chloramphenicol and [γ-^{32}P]dCTP from Amersham (Madrid, Spain). Restriction endonuclease, Klenow fragment of DNA polymerase I, and acetyl-CoA were purchased from Boehringer Mannheim.

2.2. Cell lines

PB-3c cells (kindly provided by Dr. C. Moroni, Basel Institute, Switzerland), a cloned IL-3-dependent mast cell derived from mouse bone marrow, were cultured in IMDM medium supplemented with 10% FBS (GIBCO), 100 units/ml penicillin, 1 mg/ml streptomycin, 50 μM β-mercaptoethanol, and 1% conditioned medium from X63-mIL-3 cells (kindly provided by Dr. C. Moroni). Murine EL4 thymoma cells were cultured in RPMI-1640 medium supplemented with 10% FBS, 100 units/ml penicillin, 1 mg/ml streptomycin, and 50 μM β-mercaptoethanol. The cells were cultured at 37 °C in a 5% CO_2 incubator.

2.3. Isolation of total RNA and Northern blot analysis

Total RNA was isolated from PB-3c cells using RNAzolB (Tel-Test, Frindswood, Tex, CA) according to the manufacturer's recommended procedure. Twenty micrograms of total RNA was subjected to electrophoresis through a 1% formaldehyde-agarose gel in MOPS buffer, followed by transfer to a Hybond-N nylon membrane (Amersham). Blots were prehybridized at 42 °C in SSPE hybridization solution (50% formamide, 5× Denhardt's solution, 5× SSPE, 0.1% SDS, 100 μg/ml salmon testis DNA), and probed with ^{32}P-labelled IL-3 cDNA at 45 °C in the same solution for 20 h. Equal RNA loading was determined by probing with the β-actin gene. Specific mRNA bands were quantified by scanning radioactivity using an Instant Imager (Packard, Meriden, CT).

2.4. Construction of IL-3 promoter-CAT reporter plasmids

A mouse IL-3 promoter-CAT reporter plasmid was constructed by inserting 1013 bp *Nco*I/*Sty*I promoter region (−1003 to +10 bp) into the *Hin*dIII site of the chloramphenicol acetyltransferase (CAT) reporter plasmid, pSVOCAT, and named as pIL3-1003. The 1003 bp *Nco*I/*Sty*I fragment was prepared from λMuIL-3 bacteriophage containing IL-3 genomic structure (kindly provided by Dr. K. Todokoro, RIKEN Institute, Tsukuba, Japan) (Todokoro et al., 1985). Subsequent derivatives of pIL3-1003 were constructed by inserting a 581 bp fragment of DNase I-hypersensitive (DH) site from the murine GM-CSF/IL-3 locus (Osborne et al., 1995) (pEIL3-1003) or a 281-bp *Dde*I/*Dde*I fragment from the second intron of the IL-3 gene (Todokoro et al., 1985) (pIL3-1003E) into the *Bam*HI site of the pIL3-1003 reporter plasmid. The 581 bp fragment of the DH site in the GM-CSF/IL-3 locus was prepared by PCR amplification using genomic DNA from EL4 cells, and specific primers of the DH site containing an additional *Bam*HI linker (sense: 5′-ggatccATTAAGGACGAAGGCCTC-3′ and antisense: 5′-ggatccCAAGCGTGTGGCAGGGTG-3′). The orientation of the DH site (581 bp fragment) was determined by restriction fragment analysis using restriction endonuclease.