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Fli-1 controls transcription from the MCP-1 gene promoter, which may provide a novel mechanism for chemokine and cytokine activation

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ABSTRACT

Regulation of proinflammatory cytokines and chemokines is a primary role of the innate immune response. MCP-1 is a chemokine that recruits immune cells to sites of inflammation. Expression of MCP-1 is reduced in primary kidney endothelial cells from mice with a heterozygous knockout of the Fli-1 transcription factor. Fli-1 is a member of the Ets family of transcription factors, which are evolutionarily conserved across several organisms including *Drosophilla*, *Xenopus*, mouse and human. Ets family members bind DNA through a consensus sequence GGAA/T, or Ets binding site (EBS). Fli-1 binds to EBSs within the endogenous MCP-1 promoter by ChIP assay. In this study, transient transfection assays indicate that the Fli-1 gene actively promotes transcription from the MCP-1 gene promoter in a dose-dependent manner. Mutation of the DNA binding domain of Fli-1 demonstrated that Fli-1 activates transcription of MCP-1 both directly, by binding to the promoter, and indirectly, likely through interactions with other transcription factors.

Another Ets transcription factor, Ets-1, was also tested, but failed to promote transcription. While Ets-1 failed to drive transcription independently, a weak synergistic activation of the MCP-1 promoter was observed between Ets-1 and Fli-1. In addition, Fli-1 and the NFκB family member p65 were found to interact synergistically to activate transcription from the MCP-1 promoter, while Sp1 and p50 inhibit this interaction. Deletion studies identified that EBSs in the distal and proximal MCP-1 promoter are critical for Fli-1 activation from the MCP-1 promoter. Together, these results demonstrate that Fli-1 is a novel regulator of the proinflammatory chemokine MCP-1, that interacts with other transcription factors to form a complex transcriptional mechanism for the activation of MCP-1 and mediation of the inflammatory response.

1. Introduction

Monocyte chemoattractant protein (MCP-1), also known as chemokine (C-C motif) ligand 2 (CCL2), is a chemokine family member that plays a major role in the inflammatory process. It has also been shown to be involved in immune regulation, wound healing, and act as a modulator of tumor immunity (Gu et al., 2000; Low et al., 2001; Mantovani et al., 1993; Negus et al., 1995; Monti et al., 2003). First characterized as the platelet-derived growth factor (PDGF) inducible gene *JE* (Cochran et al., 1983), both the sequence and structure of murine MCP-1 was found to be homologous to several cytokines including macrophage colony stimulating factor, IL-2, IL-6, and interferon α (Rollins et al., 1988). One of the primary roles of MCP-1 is the recruitment of monocytes, B and T lymphocytes, and natural killer cells to sites of inflammation and infection (Rollins, 1997). A wide variety of proinflammatory cytokines and chemokines, including MCP-1, have been shown to be activated through toll-like receptor (TLR) mediated stimulation of NFκB (Doyle and O'Neill, 2006). Several transcription factor binding sites have been identified in both murine and human promoter regions

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of the MCP-1 gene. Binding sites for NFκB have been mapped to distal and proximal regulatory regions within the MCP-1 promoter and an AP-1/GC box binding site that binds the transcription factor Sp1 is also located in the proximal regulatory region (Ping et al., 1996), although the proximal NFκB site was shown to be non-functional in the human promoter (Ueda et al., 1994). Transcriptional activation of both the human and murine MCP-1 promoter can be induced by TNFα (Ping et al., 1996; Ueda et al., 1994) and transcription of the human promoter has been shown to be induced by INF in astrocytes (Zhou et al., 2001). Sp1 and the NFκB transcription factor p65 produce a synergistic enhancement of the transactivation of the murine MCP-1 promoter in *Drosophila* cells and lack of the Sp1 transcription factor impairs activation and inhibits the *in vivo* assembly of the promoter/transcription factor complex (Ping et al., 2000). The regulation of MCP-1 across various cell types is complex and involves a variety of events including phosphorylation and translocation of NFκB into the nucleus, the assembly of transcription factors, and responsiveness to stimulation from other cytokines including TNFα (Ping et al., 1996).

The Ets family of transcription factors play a role in the regulation of a variety of cellular functions including: angiogenesis, apoptosis, cell proliferation, differentiation, invasiveness, immune response, lymphoid cell development, and tumor progression (Sementchenko and Watson, 2000; Hsu et al., 2004; Dittmer, 2003). They can be both positive and negative regulators of transcription and bind DNA through a winged helix-turn-helix DNA binding domain recognizing the consensus sequence GGAA/T (Sementchenko and Watson, 2000; Hsu et al., 2004; Karim et al., 1990; Findlay et al., 2013). The region surrounding the core consensus motif and cell type specificity helps to distinguish which Ets family members will bind the DNA. Ets family members may bind the same site and this redundancy may be a key aspect of their transcriptional regulation (Hsu et al., 2004). Most Ets transcription factors bind to the DNA as monomers, but interaction with other transcription factors through the pointed domain (required for protein–protein interactions) can enhance their DNA binding ability resulting in synergistic activation or repression (Li et al., 2000).

The friend leukemia insertion site 1 (Fli-1) gene is an Ets family transcription factor that is expressed in hematopoietic cells including B and T cells and vascular endothelial cells (Watson et al., 1992). Fli-1 has been implicated in the pathogenesis of both human and murine systemic lupus erythematosus (SLE) (Georgiou et al., 1996; Zhang et al., 1995a, 2004; Mathenia et al., 2010) of which glomerulonephritis, proteinuria, and autoantibody production are hallmarks (Mills, 1994; Singh and Saxena, 2009). Overexpression of Fli-1 in transgenic mice resulted in severe immune dysfunction due to an increase in infiltrating B and T lymphocytes and autoantibody production; these mice ultimately died due to tubulointerstitial nephritis and glomerulonephritis (Zhang et al., 1995b). Heterozygous expression of Fli-1 reduced autoantibody production, proteinuria, renal inflammation and necrosis, and prolonged survival (Zhang et al., 2004; Mathenia et al., 2010) in MRL/*lpr* mice and NZM2410 mice, murine models for lupus (Theofilopoulos and Dixon, 1985; Morel et al., 1994; Perry et al., 2011). Reduced proliferation of naïve B cells, independent of BCR and TLR expression in Fli-1 deficient mice has been observed (Bradshaw et al., 2008). MCP-1 has also been implicated in the development of autoimmune disease. MRL/*lpr* mice deficient in MCP-1 showed a reduction in macrophage and T cell recruitment to the kidney and glomerulus as well as reduced proteinuria, a decrease in apoptotic cells, and prolonged survival (Tesch et al., 1999). Similar results have also been observed in Fli-1$^{+/-}$ NZM2410 mice, which exhibit significantly less infiltration of inflammatory cells and decreased expression of MCP-1 in kidneys (Suzuki et al., 2012). Based on our previous results showing decreased expression of MCP-1 in kidneys and primary endothelial cells, and demonstrating that Fli-1 binds to the MCP-1 promoter (Suzuki et al., 2012); it appears that there is a direct link between Fli-1 and MCP-1 gene expression.

The goal of this study was to establish that the Fli-1 transcription factor directly regulates expression of the MCP-1 gene through transcriptional activation of the promoter. Results show that very little Fli-1 is necessary to drive transcription at a high level from the MCP-1 promoter, corroborating our previous results. Mutation of the DNA binding domain of Fli-1 demonstrated that while some of the transcriptional activation of MCP-1 was due to Fli-1 binding directly to the promoter, most of the activation was due to indirect activation of the promoter. Therefore we decided to investigate the ability of Fli-1 to interact with other transcription factors. The Ets-1 transcription factor, surprisingly, failed to drive transcription from the MCP-1 promoter. Despite the inability of Ets-1 to activate transcription on its own, when co-transfected with Fli-1 there was a slight transcriptional enhancement observed. Fli-1 and NFκB family member, p65, were found to interact to activate transcription from the MCP-1 promoter, while Sp1 and p50 inhibit this interaction. While Fli-1 is able to bind at least three cis-regulatory regions in the promoter (Suzuki et al., 2012), it appears that similar to previous studies (Ping et al., 1996; Ueda et al., 1994; Ping et al., 2000) binding sites in the distal and proximal regions are of greatest importance for transcriptional activation. Combined with our previous results, we have demonstrated that Fli-1 plays a critical role in the activation of the proinflammatory chemokine MCP-1. Thus, we have discovered that Fli-1 plays a novel role in the complex transcriptional mechanisms responsible for the activation of MCP-1 and the inflammatory response.

2. Materials and methods

2.1. Reporter and expression constructs

The full length MCP-1 promoter region was PCR amplified from the pJECAT2.6 vector (a generous gift from Dr. Jeremy Boss, Emory University School of Medicine, Atlanta, GA) with a forward primer containing the *NheI* enzyme site (underlined) and a reverse primer containing the *BglII* enzyme site (underlined). All primer sequences can be found in Table 1. The PCR program used to amplify the full length sequence was as follows: 94 °C for 3 min; thirty cycles of 94 °C for 30 s, 60 °C for 30 s, 72 °C for 1 min; 72 °C for 10 min; held at 4 °C until the program was stopped. The MCP-1 promoter region was then directionally cloned into the pGL3 basic vector upstream of the Luciferase gene (Promega, Madison, WI). Deleted portions of the MCP-1 promoter were also PCR amplified from the pJECAT2.6 vector, using the same reverse primer as the full length promoter. The forward primers used to amplify them are described in Table 1. The PCR program used was primarily the same as the one described above, except that the annealing temperature for regions B and C was 58 °C and for region D was 62 °C. All of the promoter constructs were confirmed by DNA sequencing (Genewiz, South Plainfield, NJ). The mouse Fli-1 gene containing a 5′ kozak sequence and Flag tag, was cloned into the pcDNA3.0 expression vector (Life Technologies, Grand Island, NY), which is under the control of a CMV promoter. The Ets1 cDNA was isolated from pGEM7ZEts1 through digestion with *BamHI* and *EcoRI*. The *BamHI* site was filled in and the isolated fragment was ligated into the *EcoRI* and *EcoRV* sites of the pcDNA3.0 vector. Both expression vectors have been described previously (Svenson et al., 2010). A mouse Fli-1 construct in the pSG5 expression vector (Agilent Technologies, Santa Clara, CA) that contains a single amino acid mutation to prevent DNA binding was provided by Dr. Maria Trojanowska (Boston University, School of Medicine Arthritis Center, Boston, MA) and has been described previously (Czuwara-Ladykowska et al., 2001) and for experiments with this construct, a mouse Fli-1 construct with an intact DNA