ESE-1, an Enterocyte-specific Ets Transcription Factor, Regulates MIP-3α Gene Expression in Caco-2 Human Colonic Epithelial Cells*

Received for publication, August 12, 2002, and in revised form, October 23, 2002
Published, JBC Papers in Press, October 31, 2002, DOI 10.1074/jbc.M208241200

John H. Kwon‡, Sarah Keates‡, Simos Simeonidis‡§, Franck Grall¶, Tonia A. Libermann‡, and Andrew C. Keates‡¶

From the ‡Division of Gastroenterology and §New England Baptist Bone and Joint Institute, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts 02215

We have previously shown that colonic epithelial cells are a major site of MIP-3α production in human colon and that enterocyte MIP-3α protein levels are elevated in inflammatory bowel disease. The aim of this study was to determine the molecular mechanisms regulating MIP-3α gene transcription in Caco-2 intestinal epithelial cells. We show that a κB element at nucleotides −82 to −93 of the MIP-3α promoter binds p50/p65 NF-κB heterodimers and is a major regulator of basal and interleukin-1β (IL-1β)-mediated gene activation. Scanning mutagenesis of the MIP-3α 5′-flanking region also identified two additional binding elements: Site X (nucleotides −63 to −69) and Site Y (nucleotides −143 to −154). Site X (CGCCCTTC) bound Sp1 and regulated basal MIP-3α gene transcription. Overexpression of Sp1 increased basal luciferase activity, whereas, substitutions in the Sp1 element significantly reduced reporter activity. In contrast, Site Y (AAGCAGGAATG) regulated both basal and cytokine-induced gene activation and bound the Ets nuclear factor ESE-1. Substitutions in the Site Y element markedly reduced inducible MIP-3α reporter activity. Conversely, overexpression of ESE-1 significantly up-regulated MIP-3α luciferase levels. Taken together, our findings demonstrate that co-ordinate activation and binding of ESE-1, Sp1, and NF-κB to the MIP-3α promoter is required for maximal gene expression by cytokine-stimulated Caco-2 human intestinal epithelial cells.

The chemokine superfamily of chemoattractant cytokines comprises small (8–10 kDa), inducible, pro-inflammatory proteins that specialize in mobilizing leukocytes to areas of immune challenge (1–4). Interaction of these molecules with their respective leukocyte receptors induces a characteristic set of responses that are necessary for leukocytes to leave the circulation and infiltrate tissues. These include elevation of intracellular calcium levels, modulation of adhesion molecule expression, formation of lamellipodia, and migration of leukocytes along a chemotactic gradient. Thus increased chemokine production and release is an important mechanism regulating leukocyte activation and recruitment in response to injury or infection.

To date, over 40 chemokines have been identified, which can be classified into one of four subfamilies according to the number and arrangement of conserved cysteine residues (C, CC, CXC, or CXC) (2, 5–7). Macrophage inflammatory protein-3α (also known as CCL20 or liver and activation regulated chemokine) is a recently described CC chemokine that is predominately expressed at extralymphoid sites, including the small intestine and colon and is up-regulated by pro-inflammatory stimuli (8–10). Binding of MIP-3α to its receptor, CCR6, induces migratory responses in memory CD4+ T lymphocytes and immature dendritic cells (11–14). MIP-3α also induces T-lymphocyte adhesion to the gastrointestinal-specific vascular addressin MadCAM-1 (15). In recent studies we, and others, have shown that enterocytes are a major site of MIP-3α production in human colon and that epithelial MIP-3α protein levels are elevated in IBD (15, 16). These studies are complemented by a recent report that MIP-3α mRNA levels were significantly elevated in chronically inflamed colons from IL-10 knockout mice (17). Interestingly, inhibition of colonic inflammation in this model by treatment with anti-IL-12 monoclonal antibody resulted in down-regulation of MIP-3α mRNA levels. Because Crohn’s disease in humans and the IL-10 knockout murine model of colitis are both characterized by a prominent infiltration of the colonic mucosa by CD4+ T-lymphocytes (18, 19), these data suggest that MIP-3α may play an especially important role in the recruitment of these cells to the epithelial layer during intestinal inflammation.

The production of chemokines is largely regulated at the level of gene transcription (2, 20). Of particular significance is the NF-κB/Rel family of transcription factors, which are important regulators of a variety of immune and inflammatory response genes (21–24). Several recent studies have demonstrated that MIP-3α gene expression is NF-κB-dependent (16, 25, 26). In particular, Fujii et al. (27) have reported that an NF-κB binding site in the murine MIP-3α promoter is required for cytokine-induced gene expression in Caco-2 human colonic epithelial cells. Whether production of MIP-3α by human intestinal epithelial cells requires the participation of additional nuclear factors has not been investigated. In this study we confirmed that the human MIP-3α promoter

---

* This work was supported in part by National Institutes of Health Grants DK-54920 and CA-76323 and by the Massachusetts General Hospital/Center for the Study of Inflammatory Bowel Diseases (Grant DK-43551). The costs of publication of this article were defrayed in part solely to indicate this fact.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s)AY150653.

¶ Recipient of a Research Fellowship Award from the Crohn’s and Colitis Foundation of America.

§ Recipient of a Career Development Award from the Crohn’s and Colitis Foundation of America. To whom correspondence should be addressed: Division of Gastroenterology, Dana 501, Beth Israel Deaconess Medical Center, 330 Brookline Ave., Boston, MA 02215. Tel.: 617-667-1266; Fax: 617-667-2767; E-mail: akeates@caregroup.harvard.edu.

The abbreviations used are: IBD, inflammatory bowel disease; IL, interleukin; EMSA, electrophoretic mobility shift assay; TNFα, tumor necrosis factor α; RANTES, regulated on activation normal T cell expressed and secreted.

---

"This paper is available on line at http://www.jbc.org"
NF-κB binding site plays a major role in regulating IL-1β-induced gene transcription in Caco-2 cells. We also identified two additional 5′ regulatory elements: “Site X” (nucleotides −63 to −89) and “Site Y” (nucleotides −143 to −154). Our findings demonstrate that Sp1 and the epithelium-specific Ets nuclear factor ESE-1 (binding to Site X and Site Y, respectively) are important co-regulators of MIP-3α gene expression in Caco-2 colonic epithelial cells.

MATERIALS AND METHODS

Cell Culture—Caco-2 ileocecal cells (American Type Culture Collection) were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum, 100 units/ml penicillin G sodium, 100 μg/ml streptomycin sulfate, and non-essential amino acids (Sigma) at 37 °C in an atmosphere of 5% CO2 and 95% air. For all experiments, confluent Caco-2 cell monolayers were stimulated by IL-1β (5 ng/ml). This stimulus was selected because colonic levels of this pro-inflammatory cytokine are significantly elevated in human IBD (28). Furthermore, we have shown previously that IL-1β (5 ng/ml) stimulates Caco-2 cell monolayers to secrete MIP-3α (15). However, the molecular mechanisms that control MIP-3α promoter activity are only beginning to be elucidated.

Cloning of the Human MIP-3α Promoter Region—The promoter region encoding MIP-3α mRNA was isolated from a human genomic library using a 5′-labeled MIP-3α cDNA probe. To create the probe, Caco-2 cell RNA (1 μg) was reverse-transcribed, and cDNAs encoding MIP-3α were amplified by PCR as previously described (29). PCR primers used to generate the MIP-3α probe were: sense, 5′-GAG TTT CCT GCT GCC GCC GCC TT-3′; antisense, 5′-TCT ACT GAG GAG ACC AAC AAT-3′. The genomic library screening procedure was performed essentially as described by Kentes et al. (30). Briefly, a commercially available human genomic library in Lambda Fix II (Stratagene) was plated on Escherichia coli LE 392 at a density of 37500 plaque forming units/150-mm Petri dish and then transferred to Hybond N+ membranes (Amersham Biosciences). Plaque filters were then hybridized with a random primer-labeled human MIP-3α cDNA probe at 42 °C in a solution containing 25 mM potassium phosphate (pH 7.4), 5% SSC, 5% Denhardt’s solution, 100 μg/ml denatured salmon sperm DNA, 0.1% SDS, 50% (v/v) formamide, and 10% (v/v) dextran sulfate. Final washes to remove non-hybridized probe were performed in 0.25× SSC at 42 °C. Genomic clones that hybridized with the MIP-3α probe were replated and rescreened until they were plaque-purified. Lambda DNA was then isolated from the positive clones and digested with a variety of restriction endonucleases, and Southern blots of the digests were probed with the human MIP-3α cDNA probe. Fragments of insert DNA that hybridized with the probe were then subcloned into pBluescript II (Stratagene). Genomic DNA 5′ to the MIP-3α cDNA probe was subcloned into pGEM-7 (MIP-3α promoter) by “walking” from both ends or from a defined point using specific oligonucleotide primers.

Construction of MIP-3α Luciferase Reporter Genes—An 849-bp fragment (containing nucleotides −817 to +33) of MIP-3α promoter was prepared by PCR amplification of human genomic DNA using a sense primer containing a MluI restriction site and an antisense primer containing a BglII restriction site. Following digestion with the appropriate restriction enzymes the MIP-3α promoter fragment was directionally cloned into the pGL3-Basic firefly luciferase expression vector (Promega) to generate a “full-length” MIP-3α reporter construct. Reporter genes containing sequentially truncated fragments (−712, −618, −606, −604, −557, −513, −482, −413, −376, and −234 to −53) of the MIP-3α promoter region were prepared in a similar manner using sense primers containing MluI restriction sites, and the antisense primer used to generate the full-length MIP-3α reporter construct. Mutant reporter constructs containing systematic scanning substitutions of the MIP-3α promoter or targeted substitutions in the binding elements of putative regulatory factors (i.e. NF-κB, C/EBP, Site X, and Site Y, see Table I) were prepared by oligonucleotide-directed in vitro mutagenesis as previously described (31).

Transient Transfection of Caco-2 Colonic Epithelial Cells—MIP-3α reporter constructs were transfected into Caco-2 cells using LipofectAMINE 2000 (Invitrogen) according to the manufacturer’s instructions. Cells were plated 24 h before transfection at a density of 2 × 106/well on a twelve-well tissue culture dish (Corning Costar). Three hours prior to transfection, Caco-2 cells were incubated with fetal calf serum-free, antibiotic-free media and then transfected with 1.8 μg of MIP-3α reporter gene plasmid DNA or equimolar amounts of MIP-3α reporter constructs containing truncated promoter sequences. To correct for variations in DNA uptake by the cells, each test construct was co-transfected with 0.2 μg of pRL-TK Renilla luciferase control vector (Promega). Transfections using pGL3-Basic vector without an insert were used as a negative control. For experiments investigating the effect of Sp1, Sp3, and ESE-1 overexpression on MIP-3α reporter gene activity, Caco-2 cells were co-transfected with 0.5 μg of full-length MIP-3α reporter gene and 1.5 μg of pCMV-Sp1 or pCMV-Sp3 (kindly provided by Dr. G. Suske, Marburg, Germany) or 1.5 μg of pCIG-ESE-1. After transfection for 15 h, the media were replaced and replaced with complete growth medium. After a further 24 h, Caco-2 cells were washed twice with sterile phosphate-buffered saline and incubated with serum-free medium for 16 h to reduce background luciferase levels prior to stimulation with IL-1β (5 ng/ml) for 6 h. The firefly and Renilla luciferase activities of the cells were then measured simultaneously in each sample using the Dual-Luciferase Reporter Assay System according to the manufacturer’s instructions (Promega).

Electrophoretic Mobility Shift Assay—To examine the activation of transcription factors EMSA was performed essentially as described by Keates et al. (31). Briefly, single-stranded complementary oligonucleotides bearing either wild-type or mutant MIP-3α binding elements (i.e. NF-κB, Site X, and Site Y) were prepared by custom oligonucleotide synthesis (Genosys Biotechnologies). A double-stranded oligonucleotide containing a consensus binding site for Sp1 was obtained commercially (Santa Cruz Biotechnology). After annealing, 100 ng of the double-stranded oligonucleotide was labeled using T4 polynucleotide kinase (Promega) in the presence of [γ-32P]ATP (PerkinElmer Life Sciences). Labeled probes were then purified on a Sephadex G-25 spin column (Amersham Biosciences). Binding reactions for NF-κB EMSAs were performed as described by Ferrari et al. (32). Site X EMSAs were performed according to the method of Wang et al. (33) except that 100 μM ZnSO4 was added to the binding reactions. Site Y EMSAs were performed according to the protocol of Oettgen et al. (34). Caco-2 cell nuclear extracts were preincubated for 10 min at room temperature in the reaction mixture after which the probe DNA was added and the incubation continued for another 30 min. Protein complexes were then separated from free probe using 4.5% nuclease-resistant gels. Dried gels were exposed to x-ray film at −80 °C to visualize the probe-protein complexes. To confirm the specificity of the binding reactions supershift assays were performed using antibodies to the Sp family proteins Sp1, Sp2, Sp3, and Sp4 (Santa Cruz Biotechnology), the Ets factors ESE-1 (Oncogene Research Products), ESE-2 and Ets-1 (Santa Cruz Biotechnology), and the NF-κB subunits p50, p52, p65, Rel B, and c-Rel (Santa Cruz Biotechnology). In some experiments binding specificity was also determined by competition with excess unlabeled probe. Antibodies or competing probe were added to the binding reactions at the start of the 30-min incubation period.

Data Analysis—Statistical analyses were performed using SigmaStat® for Windows® version 2.0 (Jandel Scientific Software, San Rafael, CA). Analysis of variance followed by protected t tests were used for intergroup comparisons.

RESULTS

Cloning and Analysis of the Human MIP-3α Promoter Region—Recent studies from our laboratory have shown that enterocytes are a major site of MIP-3α production in human colon and that epithelial MIP-3α protein levels are elevated in IBD (15). However, the molecular mechanisms that control MIP-3α gene expression in intestinal epithelial cells are poorly understood. To investigate the nuclear factors that regulate MIP-3α production we first cloned the 5′-flanking region of the gene from a human genomic DNA library using a MIP-3α cDNA probe. Using this approach we identified three genomic clones, designated G2-1, G6-1, G10-1, that hybridized with the MIP-3α cDNA probe from a screening of ~375,000 plaque-forming units. An 8-kb EcoRI fragment from clone G6–1 was extensively sequenced and found to contain 878 bp of the MIP-3α gene 5′-flanking region (Fig. 1). The nucleotide sequence of the MIP-3α promoter fragment was identical to that reported recently by Harant et al. (25). Further analysis of this sequence using the TransFac data base (35), identified putative binding sites for NF-κB, C/EBP, AP-1, c-Ets, and Sp1/CACCC binding protein within the first 250 bp of the promoter. A previous study by Hiershima et al. (8), using 5′ rapid amplifica-
cells were transfected with MIP-3α inducible reporter activity were also observed when Caco-2 cells. In contrast, the luciferase activity of Caco-2 cells transfected with empty expression vector was increased 11.9-fold (p<0.01) by IL-1β stimulation. The basal activity of this construct was elevated ~5.6-fold compared with Caco-2 cells transfected with empty expression vector. Compared with non-stimulated cells, increases in IL-1β-inducible reporter gene activity were also observed when Caco-2 cells were transfected with MIP-3α reporter genes containing the putative AP-1 binding site (33.6-fold, p<0.01, nucleotides –124 to +33) and the putative c-Ets binding site (54.8-fold, p<0.01, nucleotides –162 to +33). An additional increase in luciferase activity (~2.5-fold, p<0.01) was seen when Caco-2 cells were transfected with reporter genes containing nucleotides –162 to –317 of the MIP-3α promoter. However, binding elements in this region had little effect on IL-1β-induced reporter activity (~30-fold), suggesting they primarily regulate basal gene expression. Taken together, these data indicate that nuclear factor binding sites within the first 317 nucleotides of the promoter are both necessary and sufficient for basal and IL-1β-inducible MIP-3α gene expression in Caco-2 cells.

The NF-κB, but Not the C/EBP, Binding Element on the MIP-3α Promoter Is Required for Basal and IL-1β-stimulated MIP-3α Gene Expression—Previous studies have shown that the NF-κB/Rel family of transcription factors are key regulators of cDNA ends, identified a transcription initiation point 58-bp upstream from the MIP-3α start codon.

Transcription Factor Binding Sites within the First 317 Nucleotides of the MIP-3α Promoter Are Sufficient for Responses to IL-1β in Caco-2 Cells—Previous studies have shown that the production of chemokines is largely regulated via up-regulation of gene transcription (2, 20). Moreover, in a recent study we have shown that MIP-3α production by cytokine-stimulated Caco-2 cells requires de novo mRNA synthesis (15). To begin to characterize the areas of the 5′ promoter region that regulate MIP-3α gene expression, a series of luciferase reporter constructs containing sequentially truncated fragments of the MIP-3α promoter were created and transfected into Caco-2 intestinal epithelial cells (Fig. 2).

As expected, following stimulation by IL-1β (5 ng/ml, 6 h) no significant increase in luciferase reporter gene activity was seen in Caco-2 cells transfected with empty expression vector or a MIP-3α reporter gene containing the TATA binding site alone (nucleotides –62 to +33) compared with non-treated cells. In contrast, the luciferase activity of Caco-2 cells transfected with a MIP-3α reporter construct bearing putative NF-κB & C/EBP binding elements (nucleotides –103 to +33) was increased 11.9-fold (p<0.01) by IL-1β stimulation. The basal activity of this construct was elevated ~5.6-fold compared with Caco-2 cells transfected with empty expression vector. Compared with non-stimulated cells, increases in IL-1β-inducible reporter gene activity were also observed when Caco-2 cells were transfected with MIP-3α reporter genes containing the putative AP-1 binding site (33.6-fold, p<0.01, nucleotides –124 to +33) and the putative c-Ets binding site (54.8-fold, p<0.01, nucleotides –162 to +33). An additional increase in luciferase activity (~2.5-fold, p<0.01) was seen when Caco-2 cells were transfected with reporter genes containing nucleotides –162 to –317 of the MIP-3α promoter. However, binding elements in this region had little effect on IL-1β-inducible reporter activity (~30-fold), suggesting they primarily regulate basal gene expression. Taken together, these data indicate that nuclear factor binding sites within the first 317 nucleotides of the promoter are both necessary and sufficient for basal and IL-1β-inducible MIP-3α gene expression in Caco-2 cells.

The NF-κB, but Not the C/EBP, Binding Element on the MIP-3α Promoter Is Required for Basal and IL-1β-stimulated MIP-3α Gene Expression—Previous studies have shown that the NF-κB/Rel family of transcription factors are key regulators of a variety of immune and inflammatory response genes, including those of the chemokine superfamily (21–24). Consistent with these findings, our data using truncated reporter constructs suggested that the region of the MIP-3α promoter containing putative NF-κB and C/EBP binding elements was also likely to be important for the regulation of inducible gene expression in Caco-2 intestinal epithelial cells. We therefore examined this region in greater detail using mutated reporter gene constructs and EMSA (see Table I) to define the role of each nuclear factor binding site. Stimulation of Caco-2 cells transfected with the wild-type MIP-3α reporter by IL-1β (5 ng/ml, 6 h) induced an ~40-fold increase in luciferase activity compared with non-stimulated cells (Fig. 3A). Targeted substi-
tutions within the putative NF-κB binding site significantly reduced IL-1-inducible gene expression by ~90% (p < 0.01) compared with non-stimulated cells. Moreover, in non-stimulated Caco-2 cells, substitution of the putative NF-κB binding site also reduced basal luciferase activity ~50% (p < 0.01) compared with cells transfected with the wild-type MIP-3α reporter construct (Fig 3A). In contrast to these findings, substitution of the putative C/EBP binding site had no significant effect on either basal or IL-1β-mediated MIP-3α reporter gene activity. These findings demonstrate that NF-κB binding site, but not the C/EBP binding site, is an important regulator of both basal and IL-1β-inducible MIP-3α gene expression in Caco-2 intestinal epithelial cells.

To examine NF-κB regulation of MIP-3α gene expression in more detail we next performed EMSAs in non-transfected Caco-2 cells using a MIP-3α-specific probe. As expected, nuclear extracts from control Caco-2 cells showed little NF-κB binding (Fig. 3B). Within 30 min of treatment with IL-1β (5 ng/ml) Caco-2 nuclear extracts showed a marked increase in binding of two complexes to the MIP-3α-specific NF-κB probe (indicated by arrows). Levels of the faster migrating complex (band 1) appeared to remain constant over the 6-h time course of the experiment, whereas levels of the slower migrating complex (band 2) were maximal after 30 min to 1 h and declined thereafter. To determine which NF-κB subunits bound to the MIP-3α-specific probe, we next performed a supershift experiment. Nuclear extracts prepared from Caco-2 cells treated with IL-1β (5 ng/ml) for 2 h were incubated with either antibodies against the Rel proteins p50, p52, p65, Rel B, and c-rel or excess cold probe and then subjected to EMSA (Fig. 3C). The lower complex (band 1) only underwent a supershift with the p50 antibody indicating the presence of a p50/p50 homodimer. In contrast, the upper complex (band 2) was supershifted by both the p50 antibody and the p65 antibody demonstrating binding of p50/p65 heterodimers to the MIP-3α NF-κB binding element.

Scanning Mutagenesis of the MIP-3α Promoter Reveals the Presence of Multiple Binding Elements That Regulate MIP-3α Gene Expression Caco-2 Cells—As shown in Fig. 2, truncation of the MIP-3α promoter demonstrated that nuclear factor binding sites within the first 317 nucleotides of the MIP-3α promoter were essential for maximal responses to IL-1β in Caco-2 cells. Moreover, these data also indicated that other factors, in addition to NF-κB, which bind to the promoter between nucleotides −103 and −162 are required for inducible gene expression. To further characterize the factors that regulate cytokine-inducible MIP-3α gene expression we next performed scanning mutagenesis of the MIP-3α promoter between nucleotides −46 to −166 to define specific nuclear factor binding element(s). To accomplish this a series of 12 full-length MIP-3α reporter constructs containing sequential 10 bp nucleotide substitutions were prepared and transfected in Caco-2 cells (Fig. 4). As expected, the luciferase activity of Caco-2 cells transfected with reporter genes carrying mutations of the NF-κB binding site (constructs 8 and 9; nucleotides −76 to −96) were substantially

### Table I

| Substitution | NCBI Reference | Wild-type | Substitution mutant |
|--------------|----------------|-----------|---------------------|
| Site X       | −81            | ATGGGCAACACGCTTCTTGTTACA | Wild-type          |
|              | −55            | ATGGGCAACACGTTCTTGTTACA  | Substitution mutant |
| Site Y       | −166           | AATATGAGGAAAAACAGAGAAGTTTCTTT | Wild-type          |
|              | −137           | AATATGAGGAAAAACAGAATTAGTTTCTTT | Substitution mutant |

**Fig. 3.** The MIP-3α κB element binds p50/p50 and p50/p65 NF-κB and is required for basal and IL-1β-induced gene transcription in Caco-2 cells. A. Caco-2 cells were co-transfected with a wild-type MIP-3α reporter gene or mutant constructs containing targeted substitutions in either the NF-κB binding element or C/EBP binding element and the pRL-TK control vector. 48 h later the cells were treated with IL-1β (5 ng/ml) for 6 h. The activity of each construct is presented relative to the non-stimulated activity of the wild-type MIP-3α reporter gene and correct for transfection efficiency. Data are expressed as mean ± S.E. (n = 6). †, p < 0.01 versus non-stimulated activity of the wild-type construct; ††, p < 0.01 versus IL-1β-stimulated activity of the wild-type construct. B, the time course of complex binding to the κB element was investigated by EMSA using a MIP-3α-specific probe and nuclear extracts from Caco-2 cells stimulated with IL-1β (5 ng/ml). The upper portion of the autoradiogram containing the probe-protein complexes is presented. Complexes binding to the MIP-3α-specific κB probe are indicated by arrows. C, EMSA supershift study of MIP-3α-specific NF-κB activation in Caco-2 cells. Nuclear extracts from Caco-2 cells treated with IL-1β (5 ng/ml) for 2 h were incubated with either excess cold probe or antibodies against the Rel proteins p50, p52, p65, Rel B, and c-rel then subjected to EMSA. The upper portion of the autoradiogram containing the probe-protein complexes is presented. Complexes binding to the MIP-3α-specific κB probe are indicated by arrows.
activity, the ability of this construct to respond to IL-1β stimulation was similar to the wild-type construct (−10-fold). This finding suggests that binding elements located in this region primarily regulate basal MIP-3α gene expression. To examine whether one or more specific Caco-2 nuclear factors could interact with this segment of the MIP-3α promoter, a probe encompassing nucleotides −56 to −81 was synthesized and used for EMSA. As shown in Fig. 5A, two closely migrating complexes were detected in Caco-2 cell nuclear extracts using the Site X probe (designated bands 1 and 2). The binding of each complex was completely abolished in the presence of a 200-fold molar excess of unlabeled Site X probe indicating specific binding to the probe (shown in Fig. 5, C and D). Furthermore, equal levels of each complex were present in both unstimulated and in IL-1β-stimulated Caco-2 nuclear extracts consistent with our reporter gene data indicating that this region regulates basal gene expression.

To further define the Site X region, we next performed overlapping scanning mutagenesis between nucleotides −56 to −81 of the MIP-3α promoter to define a specific binding element for each complex. As shown in Fig. 5B, binding of complexes 1 and 2 was completely blocked when EMSAs were performed using Site X-scanning mutant probes 4, 5, or 6. In contrast, both complexes were able to interact (albeit to differing degrees) with Site X-scanning mutant probes 1, 2, 3, 7, and 8. These findings indicate that complexes 1 and 2 bind to the nucleotide sequence CGCCTTC in the Site X region of the MIP-3α promoter.

As part of our initial characterization of the unknown factors present in Caco-2 nuclear extracts that bind the Site X region, EMSAs were performed in the presence of the zinc-chelating agent EDTA to determine whether any of the nuclear factors were zinc finger proteins. The addition of 5 mM EDTA to the EMSA reaction mixture significantly inhibited the binding of both complexes to the Site X probe (Fig. 5C). Conversely, addition of 4 mM ZnSO₄ to binding reactions containing 5 mM EDTA restored binding of each complex to the Site X probe. These data clearly demonstrate that zinc-dependent Caco-2 nuclear factors interact with the Site X binding element on the MIP-3α promoter.

The Zinc Finger Transcription Factors Sp1 and Sp3 Bind to Site X of the MIP-3α Promoter—To determine whether any known zinc finger transcription factors could interact with Site X, this region of the MIP-3α promoter was subjected to further data base analyses using less stringent search parameters. The results of this examination suggested that Site X might contain a non-consensus binding element for the zinc finger protein Sp1. To test whether any of the complexes that bound to the Site X probe contained Sp proteins, a supershift EMSA was performed using antibodies specific for various Sp family proteins. As shown in Fig. 5D, an antibody directed against Sp1 (lane 2) was able to supershift both the upper and the lower complexes, whereas an antibody directed against Sp3 (lane 4) supershifted the lower complex only. Antibodies directed against Sp2 or Sp4 were unable to shift either complex (lanes 3 and 5). Binding of all bands to the Site X probe was reduced by an excess of unlabeled (cold) Site X probe (lane 6) indicating specific binding. Together, these data indicate that the upper Site X complex (band 1) contains Sp1, whereas the lower Site X complex (band 2) contains Sp1 and Sp3. To confirm these findings we examined the binding of each complex to Site X EMSA probes containing targeted nucleotide substitutions at positions −67 and −68. These substitutions were chosen because mutations at each of these positions have been shown previously to prevent the interaction of Sp1 with its consensus binding element (39). As shown in Fig. 5E, nucleotide substi-
The Site Y Binding Element (AAGCAGGAAGTT) Binds ESE-1, an Epithelially Expressed Ets Factor—The results of the block-scanning mutagenesis experiment presented in Fig. 4 clearly demonstrated that both basal and IL-1β-inducible reporter activity are significantly reduced when nucleotides −146 to −156 in the MIP-3α promoter are mutated. Moreover, in contrast to our findings with Site X, the level of luciferase activity following IL-1β stimulation (−5-fold) was significantly reduced compared with that seen with the wild-type construct (−10-fold). This finding suggested that Caco-2 nuclear factors interacting with Site Y regulate both basal and IL-1β-inducible MIP-3α gene expression. To characterize Site Y, we next performed an EMSA experiment to determine whether any Caco-2 nuclear factors could interact with this segment of the MIP-3α promoter. As shown in Fig. 7A, one major complex (designated band 1) was identified using the Site Y probe in Caco-2 cell nuclear extracts. Moreover, a 200-fold molar excess of unlabelled probe was able to completely prevent the formation of complexes containing the probe-protein complexes is presented. Complexes binding to the MIP-3α-specific Site X probe are indicated by arrows. B, to define the Site X binding element the MIP-3α promoter was subjected to scanning mutagenesis between nucleotides −56 and −81. EMSAs were performed using nuclear extracts from Caco-2 cells treated with IL-1β (5 ng/ml) for 2 h and a wild-type MIP-3α Site X probe or one of eight sequentially substituted Site X gel shift probes. The shaded nucleotide sequence represents the Site X binding element. C, binding of complexes to the Site X probe in the presence of EDTA or EDTA in combination with ZnSO₄ was examined by EMSA using nuclear extracts from Caco-2 cells stimulated with IL-1β (5 ng/ml) for 2 h. EMSA supershift analysis of Sp1 binding to the Site X region of the MIP-3α promoter. Nuclear extracts from Caco-2 cells treated with IL-1β (5 ng/ml) for 1 h were incubated with antibodies directed against Sp1, Sp2, Sp3, and Sp4 or with excess unlabelled consensus Sp1 oligonucleotide to compete for binding of complexes to the Site X probe was also investigated (lane 2). The shaded nucleotide sequence represents the binding site for Sp1/Sp3.

Fig. 5. Zn finger transcription factors Sp1 and Sp3 bind to the Site X region of the MIP-3α promoter. A, the time course of complex binding to Site X element was investigated by EMSA using a MIP-3α-specific probe and nuclear extracts from Caco-2 cells stimulated with IL-1β (5 ng/ml). The upper portion of the autoradiogram

tution at either of these positions completely prevented binding of both complexes to the Site X probe (lanes 3 and 4). Binding of both complexes to the wild-type probe was also substantially reduced in the presence of excess unlabelled consensus Sp1 oligonucleotide (lane 2).

Sp1, but Not Sp3, Up-regulates MIP-3α Gene Expression in Caco-2 Cells—To determine whether the Sp1/Sp3 binding element located in Site X could functionally regulate MIP-3α gene expression, we next synthesized reporter constructs carrying nucleotide substitutions at positions −67 and −68 and transfected them into Caco-2 cells. As shown in Fig. 6A, mutation of the MIP-3α promoter at either position reduced IL-1β-mediated luciferase activity by −75% (p < 0.01) compared with the wild-type control. The basal activity of either of the mutant constructs was also substantially reduced (by −50%, p < 0.01). These findings are consistent with our EMSA studies presented in Fig. 5E and demonstrate that increased MIP-3α gene expression in Caco-2 intestinal epithelial cells appears to correlate with binding of Sp1 and/or Sp3 to Site X of the promoter. To examine Sp regulation of MIP-3α gene expression further, we next investigated whether overexpression of Sp1 and/or Sp3 in Caco-2 cells could transactivate MIP-3α promoter activity. As shown in Fig. 6B, transfection of Caco-2 cells with the Sp1 expression vector (pCMV-Sp1) increased basal and IL-1β-inducible MIP-3α luciferase reporter gene activity −2-fold compared with cells transfected with the empty vector (p < 0.001 for both). In contrast, transfection of Caco-2 cells with the Sp3 expression construct (pCMV-Sp3) had little effect on basal or IL-1β-mediated MIP-3α reporter activity.

The Site Y Binding Element (AAGCAGGAAGTT) Binds ESE-1, an Epithelially Expressed Ets Factor—The results of the block-scanning mutagenesis experiment presented in Fig. 4 clearly demonstrated that both basal and IL-1β-inducible reporter activity are significantly reduced when nucleotides −146 to −156 in the MIP-3α promoter are mutated. Moreover, in contrast to our findings with Site X, the level of luciferase activity following IL-1β stimulation (−5-fold) was significantly reduced compared with that seen with the wild-type construct (−10-fold). This finding suggested that Caco-2 nuclear factors interacting with Site Y regulate both basal and IL-1β-inducible MIP-3α gene expression. To characterize Site Y, we next performed an EMSA experiment to determine whether any Caco-2 nuclear factors could interact with this segment of the MIP-3α promoter. As shown in Fig. 7A, one major complex (designated band 1) was identified using the Site Y probe in Caco-2 cell nuclear extracts. Moreover, a 200-fold molar excess of unlabelled probe was able to completely prevent the formation of complexes containing the probe-protein complexes is presented. Complexes binding to the MIP-3α-specific Site X probe are indicated by arrows. B, to define the Site X binding element the MIP-3α promoter was subjected to scanning mutagenesis between nucleotides −56 and −81. EMSAs were performed using nuclear extracts from Caco-2 cells treated with IL-1β (5 ng/ml) for 2 h and a wild-type MIP-3α Site X probe or one of eight sequentially substituted Site X gel shift probes. The shaded nucleotide sequence represents the Site X binding element. C, binding of complexes to the Site X probe in the presence of EDTA or EDTA in combination with ZnSO₄ was examined by EMSA using nuclear extracts from Caco-2 cells stimulated with IL-1β (5 ng/ml) for 2 h. EMSA supershift analysis of Sp1 binding to the Site X region of the MIP-3α promoter. Nuclear extracts from Caco-2 cells treated with IL-1β (5 ng/ml) for 1 h were incubated with antibodies directed against Sp1, Sp2, Sp3, and Sp4 or with excess unlabelled consensus Sp1 oligonucleotide to compete for binding of complexes to the Site X probe was also investigated (lane 2). The shaded nucleotide sequence represents the binding site for Sp1/Sp3.

Fig. 5. Zn finger transcription factors Sp1 and Sp3 bind to the Site X region of the MIP-3α promoter. A, the time course of complex binding to Site X element was investigated by EMSA using a MIP-3α-specific probe and nuclear extracts from Caco-2 cells stimulated with IL-1β (5 ng/ml). The upper portion of the autoradiogram...
Fig. 6. Sp1, but not Sp3, up-regulates MIP-3α gene expression in Caco-2 cells. A, to determine whether the Sp1/Sp3 binding element in Site X functionally regulates MIP-3α gene expression, Caco-2 cells were transfected with a wild-type reporter gene or mutant constructs containing targeted substitutions at positions −67 or −68 of the promoter. Caco-2 cells were also co-transfected with the pRL-TK control vector. 48 h later the cells were treated with IL-1β (5 ng/ml) for 6 h. The activity of each construct is presented relative to the non-stimulated activity of the wild-type MIP-3α reporter gene and corrected for transfection efficiency. Data are expressed as mean ± S.E. (n = 8). †††, p < 0.01 versus non-stimulated activity of the wild-type construct; ***, p < 0.001 versus IL-1β-stimulated activity of the wild-type construct. B, Caco-2 cells were co-transfected with the wild-type MIP-3α reporter gene and either a pCMV-Sp1 expression vector, a pCMV-Sp3 expression vector, or control vector (pcDNA3.1). 48 h later the cells were treated with IL-1β (5 ng/ml) for 6 h. Luciferase activities are presented relative to the non-stimulated control activity of the MIP-3α reporter gene and corrected for extract protein concentration. Data are expressed as mean ± S.E. (n = 12). ††††, p < 0.001 versus non-stimulated activity of the wild-type construct; ***, p < 0.001 versus IL-1β-stimulated activity of the wild-type construct.

this complex with the Site Y probe illustrating the specificity of the interaction (shown in Fig. 7C, lane 6). Similar to our findings with Site X, equal binding of this complex was seen in non-stimulated as well as IL-1β-stimulated Caco-2 nuclear extracts suggesting that this region regulates constitutive MIP-3α gene expression.

To characterize the Site Y region further we next subjected nucleotides −138 to −163 of the MIP-3α promoter to overlapping scanning mutagenesis to identify a specific binding element. As shown in Fig. 7B, binding of Caco-2 cell nuclear factors to Site Y was unaffected when EMSAs were performed using scanning mutant probes 1, 2, 3, or 8. In contrast, complex formation was clearly disrupted when Site Y scanning mutant oligonucleotides 4, 5, 6, or 7 were used as probes. These data demonstrate that the nucleotide sequence AAGCAAGAAGTT comprises the MIP-3α promoter Site Y binding element.

As shown in the cartoon in Fig. 2, our preliminary analysis of the MIP-3α promoter sequence using the TransFac data base (35) indicated a consensus binding motif for the nuclear factor c-Ets was located within the Site Y region at nucleotides −143 to −150. To determine whether the Caco-2 nuclear protein that bound Site Y was an Ets factor supershift EMSAs were performed. No supershifts were observed when antibodies directed against the ubiquitously expressed proteins Ets-1 (Fig. 7C, lane 3), PE-A-3 and Elk-1 (data not shown) were tested. In view of these findings, we next tested antibodies directed against three epithelially expressed members of this family: ESE-1, -2, and -3. An antibody directed against ESE-1 clearly prevented the formation of complex 1 and was accompanied by a broad supershift in the upper portion of the gel (Fig. 7C, lane 2). Antibodies directed against ESE-2 or ESE-3, however, were without effect (data not shown). To confirm this finding competition, EMSA was performed using a wild-type SPRR2A probe (which contains a previously characterized ESE-1 binding site from the SPRR2A promoter) and a mutant SPRR2A probe (which is unable bind ESE-1) (34). A 200-fold molar excess of unlabeled wild-type SPRR2A substantially reduced binding of complex 1 to the Site Y probe (lane 4). In contrast, binding this complex to the Site Y probe substituted at positions −148 and −149 (i.e. in an analogous fashion to the mutant SPRR2A probe) was also unable to compete for binding of complex 1 to the wild-type Site Y probe.

ESE-1 Up-regulates MIP-3α Gene Transcription in Caco-2 Cells—As shown in Fig. 7C, our EMSA studies indicate that the Site Y region of the MIP-3α promoter interacts with ESE-1, an epithelially expressed Ets transcription factor, in nuclear extracts prepared from Caco-2 cells. To elucidate the role ESE-1 may play in the functional regulation of MIP-3α gene expression we next synthesized a mutant reporter construct carrying targeted substitutions at positions −148 and −149, which, by EMSA analysis, was unable to bind ESE-1. As shown in Fig. 8A, in Caco-2 cells transfected with the mutant construct there was significant reduction in both basal and IL-1β-stimulated luciferase activity compared with cells transfected with the wild-type reporter gene (p < 0.001 for both). Moreover, the level of induction (i.e. -fold increase) observed with mutant construct following IL-1 stimulation (~15-fold) was substantially decreased in comparison to Caco-2 cells transfected with the control construct (~40-fold). These findings indicate binding of ESE-1 to Site Y is required for both basal and inducible MIP-3α gene expression in intestinal epithelial cells. To confirm these data, we also examined MIP-3α reporter gene activity in Caco-2 cells co-transfected with either a pCI-ESE-1 expression vector or an empty control (pCI) vector. In keeping with our other findings, overexpression of ESE-1 in Caco-2 cells significantly up-regulated basal and IL-1β-induced MIP-3α reporter activity −3-fold (p < 0.001 for both) compared with cells transfected with the pCI control construct (Fig. 8B).

DISCUSSION

In this study we demonstrate that at least three separate binding elements on the MIP-3α promoter are required to regulate MIP-3α gene expression in IL-1β-treated Caco-2 human colonic epithelial cells: an NF-κB binding site (nucleotides −82 to −93); an Sp1 site (nucleotides −63 to −69); and an ESE-1 site (nucleotides −143 to −154). Using wild-type and mutant MIP-3α reporter constructs we show that the NF-κB binding element is a major regulator of MIP-3α gene activation, because substitutions in this site substantially reduced luciferase reporter gene activity and, importantly, blocked responses to IL-1β. EMSA analysis indicated binding of p50/p65 NF-κB heterodimers to the MIP-3α κB site 30 min to 6 h after IL-1β treatment, consistent with previous reports of the kinetics of MIP-3α produc-
FIG. 7. ESE-1, an epithelially expressed member of the Ets transcription factor family, binds to the Site Y region of the MIP-3α promoter in Caco-2 cells. A, the time course of complex binding to Site Y element was investigated by EMSA using a MIP-3α-specific probe and nuclear extracts from Caco-2 cells stimulated with IL-1β (5 ng/ml). The upper portion of the autoradiogram containing the probe-protein complexes is presented. Complex binding to the MIP-3α-specific Site Y probe is indicated by an arrow. B, to define the Site Y binding element the MIP-3α promoter was subjected to scanning mutagenesis between nucleotides −137 and −163. EMSAs were performed using nuclear extracts from Caco-2 cells treated with IL-1β (5 ng/ml) for 4 h and a wild-type MIP-3α Site Y probe or one of eight sequentially substituted Site Y gel shift probes. The shaded nucleotide sequence represents the Site Y binding element. C, EMSA supershift analysis of ESE-1 binding to the Site Y region of the MIP-3α promoter. Nuclear extracts from Caco-2 cells treated with IL-1β (5 ng/ml) for 4 h were incubated with antibodies directed against ESE-1 (lane 2) or Ets-1 (lane 3) or with excess unlabeled oligonucleotides bearing the ESE-1 binding site from the SPRR2A promoter (lane 4), a mutant SPRR2A ESE-1 binding element (lane 5), the wild-type Site Y sequence (lane 6), and a mutant Site Y sequence substituted at positions −148 and −149 (lane 7). The shaded nucleotide sequence represents the binding site for ESE-1.

by Caco-2 cells. In contrast, our reporter studies indicated the Sp1 binding site appeared to primarily regulate basal MIP-3α gene transcription in Caco-2 cells. Overexpression of Sp1 increased basal luciferase activity levels, whereas, site-directed substitutions in the Sp1 element, CGCCCTTC, significantly reduced reporter activity. Similar to our findings with the NF-κB site, our reporter data suggest the ESE-1 site is required for both basal and cytokine-induced gene activation in Caco-2 cells. Overlapping scanning mutagenesis of the Site Y region demonstrated the nucleotide sequence AAGCAG-GAAGTT comprised the ESE-1 binding site. Specific mutations in this sequence markedly reduced MIP-3α gene expression. Conversely, overexpression of ESE-1 in Caco-2 cells up-regulated MIP-3α luciferase activity. Taken together, our data indicate that activation and binding of ESE-1, Sp1, and NF-κB to the MIP-3α promoter is required for maximal gene expression in cytokine-stimulated Caco-2 intestinal cells.

In keeping with several recent studies, our findings demonstrate that binding of p50/p65 NF-κB to the MIP-3α promoter is requisite for gene up-regulation. Izadpanah et al. (16) have reported that infection of HT-29 cells with an adenoviral vector encoding a mutant (non-degradable) IκBα protein can markedly inhibit cytokine-induced MIP-3α protein production. Moreover, MIP-3α gene expression in TNFα-stimulated G-361 human melanoma cells has been shown by Harant et al. (25) to require an intact NF-κB binding element. These investigators also showed increased MIP-3α reporter gene activity following overexpression of p65 NF-κB. In another study Imaizumi et al. (26) reported that Tax, a 40-kDa protein encoded by human T cell leukemia virus type-1, was capable of inducing NF-κB-dependent MIP-3α gene expression in the human T cell line JPX-9. Overexpression of Tax in JPX-9 cells was also found to induce binding of p50/p65 NF-κB to the human MIP-3α promoter as determined by EMSA. Finally, Fujiie et al. (27) have reported that an NF-κB binding site in the murine MIP-3α promoter is required for IL-1β or TNFα-induced gene expression in Caco-2 and 293T cells and that this element binds p50/p65 NF-κB heteromeric. In previous studies from our laboratory we have shown that in active inflammatory bowel disease primary colonic epithelial cell MIP-3α protein levels are significantly elevated compared with non-infamed enterocytes (15). Furthermore, increased p65 NF-κB nuclear translocation has been reported in epithelial cells and lamina propria macrophages from patients with active inflammatory bowel disease (40, 41). p65 antisense treatment can also ameliorate intestinal inflammation in mice (42). Thus, p65-containing NF-κB is likely to be an important regulator of the observed increases in enterocyte MIP-3α production in human IBD.

Several lines of evidence from the present study indicate that the Site X region of the MIP-3α promoter contains a binding site for the zinc finger nuclear factor Sp1, and that Sp1 can functionally regulate MIP-3α gene expression in Caco-2 cells. First, the EMSA supershift experiment shown in Fig. 5D clearly demonstrates that Sp1 and Sp3 in Caco-2 cell nuclear extracts can interact with a Site X-specific probe in vitro. Second, site-directed mutations in the Site X binding element that prevent binding of Sp1 and Sp3 by EMSA are associated with markedly reduced MIP-3α luciferase activity in Caco-2 cells. Finally, basal MIP-3α reporter gene activity is significantly up-regulated in Caco-2 cells overexpressing Sp1, but not Sp3.
Several reports indicate that post-translational modifications (e.g. phosphorylation and O-glycosylation) regulate both transcription and post-translational modifications of chemokine genes, including PF4, PBP, RANTES, MIP-1α, and MCP-3 (44, 53–56). The ubiquitously expressed factor Ets-1 was found to transactivate the PF4 promoter, whereas, PBP gene expression was regulated by PU.1, an Ets factor restricted primarily to cells of the immune system. For RANTES, MIP-1α, and MCP-3 the specific Ets factor regulating gene expression was not identified. Thus, to our knowledge, this is the first demonstration that ESE-1 can regulate chemokine gene expression in colonic epithelial cells or, indeed, in any other cell type.

Due to the central role of Ets proteins in hematopoietic cell development, most previous studies of ESE-1 have focused on the role played by this nuclear factor in epithelial cell differentiation and proliferation. In this context, ESE-1 has been shown to transactivate the SPRR2A-cornified envelope precursor protein promoter, the transglutaminase 3 promoter as well as the profilinigrin promoter, all of which are highly expressed during terminal keratinocyte differentiation (34, 57, 58). ESE-1 can also transactivate the Endo A/keratin 8 gene expressed primarily in simple epithelia (34). In contrast, Bremsbeck et al. (59) have reported that keratin 4 promoter activity is repressed by ESE-1 in esophageal squamous epithelial cells suggesting that this nuclear factor may have dual effects on transcription depending on the specific promoter involved. Interestingly, a recent study by Rudders et al. (60) demonstrated that ESE-1 mRNA production can be up-regulated in vascular smooth muscle cells, vascular endothelial cells, and THP-1 cells by IL-1β, TNFα, and lipopolysaccharide. These authors also showed that ESE-1 can interact directly with the p50 subunit of NF-κB to synergistically transactivate the inducible nitric-oxide synthase gene promoter. This interaction with p50 NF-κB was dependent on the presence of the ETS and A/T hook domains in ESE-1. The A/T hook domain, which is also found in IL-1β, TNFα, and lipoopolysaccharide. These authors also showed that ESE-1 can interact directly with the p50 subunit of NF-κB to synergistically transactivate the inducible nitric-oxide synthase gene promoter. This interaction with p50 NF-κB was dependent on the presence of the ETS and A/T hook domains in ESE-1. The A/T hook domain, which is also found in high mobility group proteins (e.g. HMG-I(Y)) (61, 62), is thought to alter chromatin structure through interactions with A/T-rich DNA sequences. HMG proteins are also known to facilitate gene expression via interactions with other nuclear proteins, including NF-κB and ATF-2 (63). Thus, ESE-1 may contribute to MIP-3α gene expression via A/T hook-mediated modifications to chromatin structure, the recruitment of co-activator proteins such as p50 NF-κB, or both.

A striking feature of ESE-1 expression in normal non-inflamed human colon is its predominant expression in epithelial cells (34). Similarly, recent studies from our laboratory clearly demonstrate that epithelial cells are a major site of MIP-3α production in normal colon and IBD (15). These findings suggest that expression of ESE-1 may be required for epithelial MIP-3α gene expression in vivo. Recently, the effect of targeted deletion of the ESE-1 (also designated Elf-3) in mice has been reported by Ng et al. (64). These authors found that ESE-1 deficiency was characterized by marked alterations to the architecture of the small intestine. In particular, there was poor villus formation and abnormal morphogenesis and differentiation of absorptive enterocytes and goblet cells. Surprisingly, histologic examination of colonic tissues failed to detect any gross abnormalities between wild-type and ESE-1-deficient mice. A potential explanation for this finding may be that the function of ESE-1 is compensated for by other Ets family members (e.g. ESE-3, also known as Ehf) in the colon. In this study,
however, we found no evidence that ESE-3 interacts with the Site Y region of the MIP-3alpha promoter. This is in agreement with a prior study indicating that ESE-1 and ESE-3 differentially transactivate a variety of epithelial-specific gene promoters suggesting each of these factors has distinct target gene specificities (65). In view of the emerging role of ESE-1 as a novel transcriptional mediator of immune and inflammatory responses, more exploration of the further the interactions between ESE-1, Sp1, and NF-kappaB in human colonic enterocytes and to determine whether expression of ESE-1 directs the predominant epithelial expression of MIP-3alpha in normal and inflamed human colon.

Acknowledgment—We thank Dr. Timothy C. Wang for helpful comments and suggestions.

REFERENCES

1. Springer, T. A. (1994) Cell 76, 301–314
2. Ben-Baruch, A., Michiel, D. F., and Oppenheim, J. J. (1996) J. Biol. Chem. 270, 11703–11706
3. Bagnoli, M., Dewald, B., and Moser, B. (1997) Annu. Rev. Immunol. 15, 675–705
4. Taux, D. D. (1996) Cytokine Growth Factor Rev. 7, 355–376
5. Bazan, J. F., Bacon, K. B., Hardiman, G., Wang, W., Soo, K., Rossi, D., Greaves, D. R., Zlotnik, A., and Schall, T. J. (1997) Nature 383, 640–644
6. Kelm, G. S., Kennedy, J., Bacon, K. B., Largaespada, D. A., Jenkins, N. A., Copeland, N. G., Bazan, J. F., Moore, K. W., and Schall, T. J. (1994) Science 266, 1395–1399
7. Pan, Y., Lloyd, C., Zhou, H., Dolich, S., Deeds, J., Gonzalo, J. A., Vath, J., Goselin, M., Ma, J., Dussault, B., Woolf, E., Alperin, G., Culpepper, J., Gutierrez-Ramos, J. C., and Gearing, D. (1997) Nature 387, 611–617
8. Hieshima, K., Imai, T., Opdenakker, G., Van Damme, J., Kusuda, J., Tei, H., Sakai, Y., Tekatsumi, K., Miura, R., Yoshie, O., and Nomiyama, H. (1997) J. Biol. Chem. 272, 5846–5853
9. Rossi, D. L., Vicari, A. P., Franco-Bacon, K., McIlhanan, T. K., and Zlotnik, A. (1987) J. Immunol. 158, 1033–1038
10. Tanaka, Y., Imai, T., Baba, M., Ishikawa, I., Uehira, M., and Nomiyama, H. (1997) J. Biol. Chem. 272, 3021–3027
ESE-1, an Enterocyte-specific Ets Transcription Factor, Regulates MIP-3α Gene Expression in Caco-2 Human Colonic Epithelial Cells
John H. Kwon, Sarah Keates, Simos Simeonidis, Franck Grall, Towia A. Libermann and Andrew C. Keates

J. Biol. Chem. 2003, 278:875-884.
doi: 10.1074/jbc.M208241200 originally published online October 31, 2002

Access the most updated version of this article at doi: 10.1074/jbc.M208241200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 65 references, 29 of which can be accessed free at http://www.jbc.org/content/278/2/875.full.html#ref-list-1