Multiple Ets Factors and Interferon Regulatory Factor-4 Modulate CD68 Expression in a Cell Type-specific Manner*

Dawn O'Reilly, Carmel M. Quinn, Tariq El-Shanawany, Siamon Gordon, and David R. Greaves‡

From the Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford OX1 3RE, United Kingdom

Received for publication, December 1, 2002, and in revised form, March 13, 2003
Published, JBC Papers in Press, April 3, 2003, DOI 10.1074/jbc.M212150200

CD68 is a transmembrane glycoprotein expressed in all cells of the mononuclear phagocyte lineage including monocytes and tissue resident macrophages. Deletion analysis of the 5′-flanking sequences of the gene demonstrated that the proximal −150-bp sequence of the CD68 promoter exhibits high level promoter activity in macrophages. Mutations that abolish Ets factor binding at positions −106 and −89 reduce promoter activity in macrophages to 12 and 30%, respectively. Band shift experiments show that PU.1 associates with the −89 site whereas, Elf-1 preferentially binds the −106 Ets binding site and enhances CD68 activity in vitro. Furthermore, chromatin immunoprecipitation experiments confirm that Elf-1 and PU.1 associate with the CD68 proximal promoter in vivo in THP-1 cells. PU.1 does not bind to the proximal promoter alone but instead forms heterocomplexes with members of the interferon regulatory factor family (IRF) including IRF-4 and IRF-8. IRF-4 and IRF-8 typically mediate transcriptional activation when associated with PU.1 on composite elements. However, our data show that PU.1/IRF-4 and IRF-8 heterocomplexes down-regulate CD68 promoter activity in macrophages and repression is dependent on the integrity of both the IRF and PU.1 half-sites of this composite element. Chromatin immunoprecipitation data reveal that neither IRF-4 nor IRF-8 associate with the CD68 proximal promoter in macrophages in vitro but IRF-4 is associated with the promoter in B lymphocytes. We propose that expression of CD68 in myeloid cells requires the Ets transcription factors Elf-1 and PU.1 and CD68 expression is down-regulated in lymphoid cells by combinatorial interactions between PU.1 and IRF-4.

Monoclonal antibodies of the CD68 cluster show strong staining of all monocytes and macrophages in normal human tissues and CD68 is widely regarded as a pan macrophage marker in immunohistochemistry studies (1, 2). The protein recognized by CD68 antibodies is a member of the lysosome-associated membrane protein family (3). Human CD68 shows 72% identity with the murine macroislin protein and displays the same overall organization with an N-terminal 117-amino acid mucin domain separated from a 165-amino acid lysosome-associated membrane protein domain by a 13-amino acid proline-rich spacer region (4–6). Its function, as yet, has not been established but CD68 and macroislin have been shown to bind and uptake oxidized low density lipoproteins, which are accumulated in macrophage-derived foam cells within atherosclerotic plaques (7–9).

CD68 is one of 9 genes in a 140-kb region of the human chromosome 17p13 (10). It lies 669 bp 3′ to the human eukaryotic initiation factor 4A1 gene and 5′ to a MDPU1 gene, which encodes a repressor of the Lec 15 and Lec 35 glycosylation mutants of Chinese hamster ovary cells. All of the genes clustered around CD68 have different expression profiles, being expressed in different tissues with different developmental specificities. Its close proximity, however, to a housekeeping gene, EIF-4A1, suggests that the CD68 gene is likely to be in a permanently open chromatin configuration (11). We have shown that the first intron of the EIF-4A1 gene contains a methylation free island, which can act as a potent enhancer in a wide range of cell types (12). Thus, the CD68 gene may not need a specific chromatin opening region but rather only genetic elements, which act to restrict expression to macrophages. The need to identify such genetic regulatory elements to target transgene expression to macrophages led us to clone and characterize the CD68 promoter.

Our studies show that sequences between −150 and +2 of the human CD68 gene give maximal promoter activity in transiently transfected macrophage cell lines and that multiple Ets factors bind this promoter in vitro and in vivo. An important element of the promoter is a composite PU.1/IRF site that binds PU.1 and interferon regulatory factor members IRF-4† and IRF-8. Although both factors are able to repress CD68 promoter activity in a DNA-dependent manner in vitro in THP-1 cells, only IRF-4 is associated with the promoter in vivo in B lymphocytes. We propose that CD68 expression is regulated in different cell types by specific activators and repressors of transcription including Ets family members and IRF-4.

EXPERIMENTAL PROCEDURES

RNA Preparation and 5′ RACE Analysis of CD68 mRNA

Total RNA was prepared from human peripheral blood-derived macrophages by guanidium/phenol extraction (RNAzol-B, Biogenesis). Rapid amplification of cDNA 5′ ends (5′ RACE) was performed using a 5′ RACE system (Invitrogen). Reverse transcription of 1 μg of human macrophage total RNA was performed using the primer CD68GSP1 (GCTATTTGCTTTGGTGATG). Tailed first strand cDNA was used as a template for PCR with a 5′ anchor primer (5′-CUACUCAUCUAUGCC-CACCGTGACTAGTACGGIGIIGGGIIG) and the CD68GSP2 primer (5′-TTCAGTGTCCCTCTGTAACC). Gel purified PCR products were cloned via overhangin 3′ A/A residues and sequenced with fluorescently labeled PAGE by the method of Page (13). This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† British Heart Foundation Basic Science Lecturer. To whom correspondence should be addressed: Sir William Dunn School of Pathology, South Parks Road, Oxford OX1 3RE, United Kingdom. Tel.: 44-1865-285519; Fax: 44-1865-275515; E-mail: david.greaves@path.ox.ac.uk.

‡ This work was supported by grants from the British Heart Foundation and Glaxo Wellcome. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The abbreviations used are: IRF, interferon regulator factor; RACE, rapid amplification of cDNA ends; MEF, myeloid Elf-1-like factor; FCS, fetal calf serum; PBS, phosphate-buffered saline; CAT, chloramphenicol acetytransferase; EMSA, electrophoretic mobility shift assay; IL, interleukin; UTR, untranslated region; TLR, Toll-like receptor.
Macrophase-specific Expression of CD68

recently labeled deoxyribonucleotides and Taq FS DNA polymerase (ABI Prism FS).

Construction of Promoter Reporter Plasmids

A 2940-bp BstXI fragment was purified from cosmID CD68C1 (10). The BstXI fragment was rendered blunt ended by incubation with T4 DNA polymerase and cloned into the EcoRV site of plasmid pSK-Stratagene, La Jolla, CA) to give plasmid pCD68Bst3-2. The 3' BstXI site contains the CD68 ATG initiation codon, which was removed by the 3'-endase activity of T4 DNA polymerase. A HindIII-XhoI fragment containing 2940 bp of DNA upstream of the CD68 ATG codon was cloned into the reporter vector pCAT Basic (Promega) to give the plasmid 2940CDE68PCAT. Plasmid 2940CDE68PCAT was digested with SstI to give the plasmid 951CDE68PCat. All other CD68 promoter deletions in pCAT Basic were prepared by PCR using plasmid 2940CDE68PCAT as a template and 5' and 3' oligonucleotide primers that added a HindIII site and a common 3' primer that spanned the XhoI cloning site of plasmid 2940CDE68PCat. The 150 to 12 CD68 promoter fragment was PCR amplified and cloned between the KpnI and Xhol sites of the luciferase reporter vector pGL3 Basic (Promega) to give the plasmid 150CD68Luc. A 5' deletion series of the CD68 promoter in pGL3 Basic was made using PCR and primers that added a 5' MluI site. Linker scanning mutations were made by cloning PCR products extending from 150 to the 5' deletion end point between KpnI and MluI sites of 5' deletion clones. Double point mutations in the Ets binding site and the PU.1/I F composite site were generated using the Transformer Site-directed mutagenesis kit (Clontech) and the -150CD68luc DNA was used as template. Two oligonucleotides were generated to mutate the PU.1/I F composite element. The 89mIRF oligo contains a mutation within the IRF binding site and the -89mIRF contains a mutation at the PU.1/I F binding site. Deleted mice were crossed with mice deficient in Ets factors. Ets factors were expressed in vitro in transfected cells either in the absence or presence of heat-treated cell lysates (65 °C, 20 min) which had been pre-warmed to 37 °C. The enzyme activity was determined by spectrophotometry at 570 nm. CAT enzyme activity was measured by enzyme-linked immunosorbent assay of cell lysates (65 °C, 20 min) which had been pre-warmed to 37 °C (14). A CAT enzyme standard curve was generated using dilutions of purified CAT enzyme (Promega) in each experiment and all CAT enzyme assays were within the linear range of the enzyme assay. Transfected cell lysates were prepared in reporter lysis buffer and CAT enzyme activities were determined using a Firefly luciferase assay kit (Promega).

Transfection Factor Expression Vectors and in Vivo Protein Synthesis

Mammalian expression vectors containing murine PU.1 and human Elf-1 cDNAs were kind gifts of Professor Celeste Simon. The plasmid pCDNA3 Sp-C was a gift of Dr. R. Carlson and a cDNA encoding murine Fl-1 was a gift of Dr. Jon Frampton. MEF expression vector was provided by Dr. Stephen Nimeroff. All Ets factor coding cDNAs were cloned into the mammalian expression vector pCDNA3 (Promega) to facilitate comparative analyses. Ets factors were expressed in vitro using the Tnt coupled reticulocyte lysate system (Promega). Murine IRF-4 cloned into pRc/CMV was a kind gift from Dr. Harinder Singh and murine IRF-8 in pTARGET vector was a gift of Dr. Ben-Zion Levi. Both genes were subcloned into pCDNA3.

Mammalian Cell Culture and Transient Transfection—The murine macrophage cell lines RAW264.7 and P88.D1, the murine B cell line A20, the human monocytic leukemia cell line THP-1, the human myeloid cell lines HL60 and U937, and the human B lymphomas Raji and Thiel (kind gift from Dr. David Mason) cells were maintained in RMPI 1640 (Invitrogen) supplemented with 10% heat inactivated fetal calf serum (FCS) (Sigma), 100 units ml-1 penicillin, 100 μg ml-1 streptomycin, and 2 μg ml-1 gentamycin. COS-7 cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% FCS, antibiotics and glutamine, and HEK293, HepG2, and NIH 3T3 cells were grown in minimal essential medium supplemented with 10% FCS, 1% nonessential amino acids, antibiotics, and 10% FCS. RAW264.7 and P88.D1 cells were grown to confluence, harvested, and washed once in phosphate-buffered saline (PBS), and resuspended in Opti-MEM I serum-free medium (Invitrogen) for RAW cells or RPMI 1640 (no FCS) for P88.D1 cells. Aliquots of 2 × 10^7 cells (0.5 ml) were mixed with 20 μg of pcAT or 10 μg of luciferase reporter plasmid DNA and 2 μg of pCDNA3 β-galactosidase reporter DNA, added to a 0.4-cm electrode gap electroporation cuvette (Bio-Rad) and shocked in a Bio Rad GenePulser (280 V, 1090 microfarads) at room temperature. Cells were recovered immediately into 10 ml of cell growth medium that had been pre-warmed to 37 °C. Transient transfection efficiencies between 10 and 20% were routinely obtained with P388.D1 and RAW cells. THP-1 transfections were performed using GeneJuice transfection reagent (Novagen) in 24-well plates, according to the manufacturer's instructions. Briefly, the day of transfection, cells in mid-log phase were plated at 1 × 10^6 ml-1 in 1 ml of complete RPMI 1640 media. 100 μl of plasmid/GeneJuice in Opti-MEM I (1 μg of DNA, 4 μg of GeneJuice) was added directly to the cells. After 16–24 h incubation the cells were washed twice in PBS and harvested into 1× luciferase buffer (Promega). HepG2, NIH 3T3, HEK293, and COS-7 cells were grown to 70–80% confluence in 6-well Costar dishes, washed twice with Opti-MEM I before addition of 2.5 ml of plasmid DNA-cationic lipid complex (2 μg of DNA, 25 μg of LipofectAMINE (Invitrogen) in Opti-MEM I). After 6–18 h incubation the medium was aspirated, cells were washed once in PBS, and resuspended into complete medium for 24–36 h before analysis. HepG2, NIH 3T3, COS-7, and HEK293 cell transient transfection efficiencies were in excess of 40% as judged by co-transfection with a green fluorescent protein reporter plasmid. Transient transfection in these cells were also performed using GeneJuice reagent following the manufacturer’s instructions for adherent cells.

Reporter Gene Assays—Transfected cells were washed once with PBS and harvested by scraping into PBS. Cell pellets were resuspended in 100 μl of 0.25% Triton-HCl (pH 7.8) and subjected to three rounds of freeze-thaw lysis. Cell lysates were assayed for β-galactosidase enzyme activity using the colorimetric substrate chlorophenol red-β-D-galacto-pyranoside (Roche Diagnostics) in a 96-well plate assay in 50 mM potassium phosphate, pH 7.0 with 0.2 mM beta mercaptoethanol. β-galactosidase activity was determined by spectrophotometry at 570 nm. CAT enzyme activity was measured by enzyme-linked immunosorbent assay of cell lysates (65 °C, 20 min) which had been pre-warmed to 37 °C (14). A CAT enzyme standard curve was generated using dilutions of purified CAT enzyme (Promega) in each experiment and all CAT enzyme assays were within the linear range of the enzyme assay. Transfected cell lysates were prepared in reporter lysis buffer and CAT enzyme activities were determined using a Firefly luciferase assay kit (Promega).

Electrophoretic Mobility Shift Assays (EMSA), Quantitation, and Western Blotting

Annealed oligonucleotide probes (0.5 pmol) labeled with 32P were incubated with 5–15 μg of nuclear extract in Buffer C (40 mM Hepes-KOH, pH 7.9, 12.5% glycerol, 1 mM EDTA, 0.1 mg ml-1 bovine serum albumin, 50 μg ml-1 poly(dI-dC), 50 μg ml-1 salmon sperm DNA) for 30 min at room temperature. The sequence of the oligonucleotides corresponding to the transcription factor binding sites were: Ets binding site, 5’-CGAATTCCTCCTCTCCCTTCTTCCAAGG-3’; mutated IRF binding site: 5’-GACTTTCATTTGGTC-3’; PU.1/I F composite element: 5’-GACTTTCATTTGGTC-3’; mutated PU.1/I F binding site: 5’-GACTTTCATTTGGTC-3’. After 6–18 h incubation the medium was aspirated, cells were washed once in PBS, and resuspended into complete medium for 24–36 h before analysis. HepG2, NIH 3T3, COS-7, and HEK293 cell transient transfection efficiencies were in excess of 40% as judged by co-transfection with a green fluorescent protein reporter plasmid. Transient transfection in these cells were also performed using GeneJuice reagent following the manufacturer’s instructions for adherent cells.
of Taylor et al. (15). Briefly, spleenocytes were harvested by standard methods using a combination of digestion with Liberase Blendzyme II in RPMI (Roche Diagnostics) and mechanical dissociation. Enzymatic activity was quenched with RPMI, 20% FCS, erythrocytes were lysed with Gey’s solution, and cell debris was removed by centrifugation through 100% FCS at 1000 rpm.

**Lymphocyte Isolation and Positive Selection of Human Primary B Cells**

Lymphocytes were isolated using Ficoll-Plaque (Amersham Biosciences) density gradients. Briefly, buffy coats were diluted 1:3 in PBS and layered onto Ficoll-Plaque, at a ratio of blood:Ficoll of 2.5:1. Supernatants were prepared for 20 min on ice. Nuclei were pelleted at 4 °C for 2 h. Supernatants were diluted 1:5 with buffer C (0.01% protease A buffer plus 0.2 mg ml⁻¹ proteinase A (Roche Diagnostics) and incubated at 45 °C for 2 h. Aprotinin, 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻¹ trypsin inhibitor, and 1 μg ml⁻1
The level of expression obtained with this fragment of the CD68 promoter is twice that obtained with the SV40 enhancer/promoter (pCAT Control) cloned in the same reporter gene vector (pCAT Basic). Deletion of sequences between \(-150\) and \(-119\) led to a slight decrease in CD68 promoter activity, whereas deletion of sequences between \(-119\) and \(-96\) virtually abolish promoter activity. In transient transfection assays using the \(-150\) CD68 construct significant levels of CD68 promoter activity were seen in the non-macrophage cell lines tested (Fig. 2C). Promoter activity was reduced by 70–82% in 293 and NIH 3T3 cells, respectively, and as much as 95% in HepG2 cells as compared with values obtained with macro-
phage cells. From these experiments we conclude that sequences between −143 and +2 give maximal expression in macrophages.

**The Proximal Promoter of the CD68 Gene Contains Several Purine-rich Motifs**—Closer analysis of the proximal promoter region revealed a number of purine-rich motifs on either strand at positions −106, −89, and −78 with the consensus core 5′-GGAA-3′ sequence (Fig. 2A). This sequence motif is recognized by transcription factors of the Ets family including PU.1, a factor known to be required for optimal expression of a number of myeloid-specific genes (20–23). To determine whether these binding sites were functional we cloned the CD68 proximal promoter, −150 to +2 sequence, upstream of the luciferase reporter gene in plasmid pGL3 Basic and generated a series of linker scan and point mutations to alter the consensus Ets core sequence, GGAA or TTCC on the reverse strand. Transient transfection experiments were performed in THP-1 cells and luciferase activities were normalized by co-transfection with a β-galactosidase expressing vector (CMVβ-gal). Mutating the GGAA-containing sequence at −106, either by replacing 6 core nucleotides with an MluI site or making a double point mutation, has the most deleterious effect on CD68 promoter activity, giving only 20% of wild type luciferase expression (Fig. 2, D and E). Mutating the −89 site reduces promoter activity by up to 60–70% and mutating −78 has little or no effect (Fig. 2, D and E). These data indicate that the potential Ets binding sites at positions −106 and −89 are required for maximal promoter activity in macrophages.

**Multiple Ets Factors Bind the Proximal CD68 Promoter with Different Affinities**—Point mutations of the CD68 promoter showed that two of the three GGAA sequences between −150 and +2 potentially contribute to CD68 promoter activity. To test whether Ets factors can bind to these sites we performed EMSAs. Nuclear extracts prepared from THP-1 cells and in vitro translated Ets factors were incubated with double-stranded radiolabeled oligonucleotides corresponding to the purine-rich sequences at −106 and −89. As shown in Fig. 3A, we observed three specific protein-DNA complexes on the −106 CD68 probe (designated I, II, and III). Complex III co-migrates with in vitro translated PU.1 and is shifted with antibodies specific to PU.1. Complex II co-migrates with in vitro translated Fli-1 and this minor complex is specifically supershifted by addition of an anti-Fli-1 antiserum. The slowest and the strongest of the three complexes. Complex I co-migrates with in vitro translated Elf-1 and MEF. This complex contains Elf-1 as judged by antibody supershift with an anti-Elf-1 antibody. Higher concentrations of antibody completely supershifted this complex whereas antibodies to MEF failed to do so (data not shown).

The −89 CD68 probe gives a different pattern of factor binding (designated IV, V, and VI) (Fig. 3A). Antibody supershift experiments characterized the slower migrating complex IV as Elf-1 and the two other complexes (V and VI) contain PU.1, and as both are supershifted with anti-PU.1-specific antiserum and remain unchanged with the addition of a Fli-1-specific antiserum Complex VI, co-migrates with in vitro translated PU.1 and the difference in mobility with complex V suggests the presence of an additional factor that interacts with PU.1 at this site. Interestingly, high concentrations of THP-1 extract (up to 12 μg) are necessary to see this complex, which suggests that this additional factor may not be very abundant in THP-1 cells or weakly associates with this site. All of these complexes were successfully competed for by cold −106 CD68 and −89 CD68 probes, respectively, whereas none of the binding activities were competed for by oligonucleotides mutated within the GGAA core. The −89 CD68 probe also contains the −78 GGAA sequence. To rule out that complexes IV, V, or VI are forming on this 3′ site we tested probes mutated at −89 and −78. Ets factor binding activities were abolished by mutation of the −89 TTCC sequence, but unaffected by mutation of the −78 sequence (data not shown).

We used competition experiments and phosphorimaging analysis to estimate the relative affinities of the CD68 GGAA sequences for the different Ets factors. EMSA experiments were performed with 0.5 pmol of labeled −89 CD68 probe and THP-1 nuclear extract with increasing amounts of unlabeled −106 CD68 or −89 CD68 oligonucleotides as shown in Fig. 3B. All three EMSA complexes appear to be competed equally with increasing concentrations of cold −106 CD68 probe (Fig. 3B, left panel). However, quantitation analysis reveal that at least 6.5-fold (16 pmol) more cold competitor is required to reduce PU.1 binding by 50% as compared with Elf-1 and Fli-1 (2.5 pmol) (Fig. 3C, right panel). Although the −106 Ets binding site has a greater affinity for all three Ets factors as compared with the −89 site, Elf-1 and Fli-1 bind preferentially to this site, and PU.1 favors the TTCC sequence at position −89.

**Multiple Ets Factors Regulate CD68 Proximal Promoter Activity in Vitro and Associate with the Promoter in Vivo**—To determine which, if any, of the Ets factors bound to the −106 and −89 Ets binding sites could regulate the CD68 promoter activity, transactivation experiments were performed in COS-7 cells. All Ets factor cDNAs were subcloned into the same mammalian expression vector (pcDNA3) to facilitate comparative analyses. The −150CD68lac construct was co-transfected with Elf-1, Fli-1, Spi-C, and PU.1 expression constructs. As shown in Fig. 4A, Elf-1 enhanced CD68 promoter activity 4-fold and Fli-1 enhanced expression 6-fold. We consistently observed that the Ets factors Spi-C and PU.1 did not transactivate the CD68 promoter. We performed chromatin immunoprecipitation analysis to determine whether these Ets factors are indeed bound to the CD68 proximal promoter in macrophages in vivo. THP-1 cells were cross-linked as described under “Experimental Procedures” and protein-DNA complexes were immunoprecipitated with specific antibodies to Elf-1, Fli-1, PU.1, and an IgG control. Precipitated DNA was used as template for PCR amplification with genomic primers specific for the proximal promoter region of the CD68 gene. As a control for antibody specificity we used primers directed to the CD68 3′-UTR. Fig. 4B shows a schematic representation of the CD68 gene and the regions amplified by the CD68 promoter and 3′-UTR primer pairs. Buffer alone and no antibody control reactions were also prepared to control for PCR specificity. The data of Fig. 4C show that PU.1, Fli-1, and Elf-1 are all associated with the proximal promoter of the CD68 gene in THP-1 cells. The PCR primers specific for this region produced a signal of predicted size (318 bp) from the total input DNA and the anti-Elf-1, anti-Fli-1, and anti-PU.1 immunoprecipitations. Unexpectedly, PCR primers specific for the 3′-UTR of the CD68 gene produced an amplicon of the predicted size from the anti-Fli-1 immunoprecipitation. Analysis of this region indicates that there are no consensus Ets binding sites. However, it is possible that Fli-1 associates with the DNA at a non-canonical binding site or that it binds indirectly to the 3′-UTR by associating with a factor bound to a different sequence element. Anti-MEF immunoprecipitations failed to give a signal in the same assay (data not shown). These data show that multiple Ets factors can transactivate the CD68 proximal promoter in vitro and bind the CD68 promoter in vivo.
Identification of the PU.1 Interacting Factor Bound to the CD68 Purine-rich Motif

To characterize the PU.1-interacting factor bound to the CD68 probe (complex V, Fig. 3A) we analyzed the sequence further and found a possible binding site for members of the IRF family, just 5' of the GGAA core sequence. Sequence comparison of this putative PU.1/IRF binding site concentration (0.3 pmol) of labeled -106 CD68 probe was incubated with 3-fold increasing concentrations of unlabeled -106 CD68 probe (left panel) or unlabeled -89 CD68 probe (right panel). Maximum concentration of cold competitor was 40 pmol. C, the gel from B above was scanned using a PhosphorImager and the intensity of each complex was quantitated using ImageQuant software. The results are plotted as relative inhibition of binding against picomoles of cold competitor. The data shown in B and C are from a single experiment but were reproducible in three independent experiments.

**Fig. 3.** Multiple Ets factors bind the purine-rich motifs within the CD68 proximal promoter with different specificities. A, double-stranded oligonucleotides containing the potential Ets binding sites at -106 and -89 were 5' labeled with 32P and used in EMSA with THP-1 nuclear extracts and in vitro translated Ets factors as indicated. Supershift experiments with antisera against Ets family transcription factors are indicated above each lane. Complexes bound to the -106 CD68 probe are labeled I, II, and III and to the -89 CD68 probe are denoted IV, V, and VI. A double-stranded probe mutated at the -89 site but leaving the -78 site intact failed to bind any complexes with THP-1 nuclear extracts (data not shown). The - denotes probe alone. B, a fixed concentration (0.3 pmol) of labeled -106 CD68 probe was incubated with 3-fold increasing concentrations of unlabeled -106 CD68 probe (left panel) or unlabeled -89 CD68 probe (right panel). Maximum concentration of cold competitor was 40 pmol. C, the gel from B above was scanned using a PhosphorImager and the intensity of each complex was quantitated using ImageQuant software. The results are plotted as relative inhibition of binding against picomoles of cold competitor. The data shown in B and C are from a single experiment but were reproducible in three independent experiments.

**Fig. 4.** Multiple Ets factors can induce the proximal CD68 promoter in vitro and associate with the endogenous promoter in macrophages in vivo. A, the -150CD68luc construct was transfected into COS-7 cells with a control plasmid (pcDNA3) and Ets factor expression vectors as indicated. Cell lysates were prepared 48 h post-transfection and relative luciferase activity is represented as -fold luciferase activity in the absence of Ets factor binding. Mean values are shown; error bars represent the S.D. of two independent experiments. B, schematic representation of the CD68 gene. The numbers denote the intron/exon boundaries and 5' and 3' UTRs. The solid lines indicate the region amplified by the primer pairs used in the chromatin immunoprecipitation assay in C. C, formaldehyde cross-linked chromatin from THP-1 cells was incubated with antisera specific to Elf-1, PU.1, Fli-1, and an IgG control and analyzed as described under "Experimental Procedures." Buffer alone (Mock), no antibody (No Ab.), and total chromatin input (Total) were also examined. Primers to the proximal promoter and 3' UTR of the human CD68 gene were used for PCR analysis as indicated in B.
ing motif with known PU.1/IRF composite elements revealed strong sequence similarity (Table I). There are 10 IRF family members characterized to date with IRF-4 (Pip/LSIRF/ICSAT) and IRF-8 (ICSBP) primarily restricted to lymphoid and myeloid cells (24–27). Interactions between PU.1 and these two IRF members have been observed previously (28–31). As shown in Fig. 5A three complexes were observed with THP-1 extracts incubated with radiolabeled −89 CD68 probe, as expected. Antibodies against both IRF-4 and IRF-8 completely shift complex V, whereas antisera to other IRF family members including IRF-1 and IRF-2 fail to do so. Antibody specificity problems have been reported with anti-IRF-4 and anti-IRF-8 antisera in gel shift assays. In particular, Rehl et al. (32) previously demonstrated that the IRF-4 specific antisera cross-reacts with in vitro translated IRF-8 protein and likewise the IRF-8 antisera supershifted an IRF-4/IRF-2 complex bound to an interferon-stimulated response element using a B cell nuclear extract isolated from an IRF-8 knockout mouse (33). Hence, it is difficult to conclude which of the two factors are present in this complex V. As expected, anti-PU.1 antisera supershifted PU.1 and either PU.1/IRF-4 or PU.1/IRF-8 hetero complexes, suggesting that neither IRF-4 nor IRF-8 could bind to the DNA in the absence of PU.1.

To show that the PU.1/IRF complex formed on the −89 CD68 probe depended on the binding of IRF-4 or IRF-8 to the sequence 5′ of the PU.1 site, we introduced point mutations into the IRF binding site leaving the PU.1 site intact. Mutation of the IRF binding site completely abolished PU.1/IRF binding but does not affect the binding of either Elf-1 or PU.1 alone (Fig. 5D). In summary, we have identified a composite binding element for PU.1/IRF factors on the CD68 promoter and shown that DNA-dependent binding of IRF-4 or IRF-8 requires association with PU.1.

**TABLE I**

| Gene | PU.1/IRF composite element |
|------|-----------------------------|
| Consensus sequence | RRRGAAGTGAAANY |
| Hu CD68* | GAGGAAAGAAG |
| Mu CD68* | GAGGAAAGAAG |
| Hu gp91phox | GAGGAAGAAAC |
| Hu Igk 3 enhancer | GAGGAACGAGAAAC |
| Hu TLR-4* | GAGGAAGAAGAAC |
| Hu CD20 | GAGGAAGAAGAAG |
| Hu IL-1β* | GAGGAAGAAGAAG |

* Opposite strand.

50–60% of wild type, when the PU.1 binding site is mutated. We know from EMSA data that IRF-4 cannot associate with the DNA in the absence of PU.1 (Fig. 5, lane 4). However, IRF-4 may still be able to weakly associate with the DNA and exert its activity in the presence of PU.1 even when this factor cannot itself bind to the DNA at this site. Also, although Elf-1 preferentially associates with the upstream −106 site PU.1 can also bind this site (Fig. 3, A–C) and it is possible that IRF-4 may exert its repression on the CD68 promoter via interactions with PU.1 bound to the −106 site when the −89 site is no longer available. EMSA supershift data (Fig. 5A) showed that IRF-8 was equally likely to associate with the PU.1/IRF composite site. As observed with IRF-4, overexpression of IRF-8 in THP-1 cells reduces CD68 reporter activity by 60–70% of wild type (Fig. 6C). Mutation of either the IRF site or the PU.1 site also abolishes this repression. Our data suggest that IRF-4 and IRF-8 must contact the DNA for transcriptional repression to occur and that both factors require association with PU.1 to exert this activity.

**DIFFERENTIAL EXPRESS**

**ION OF IRF-4 AND IRF-8 IN MACROPHAGES—**CD68 is expressed mainly in cells of the mononuclear phagocytic lineage. Since both IRF-4 and IRF-8 appear to repress CD68 promoter activity we sought to determine whether either of these factors are expressed in cell lines that do not produce any detectable CD68 protein. Western blotting experiments were performed on nuclear lysates from monocyte cell lines including THP-1, U937, and HL60, B lymphocytic cell

**FIG. 5.** PU.1 interacts with members of the IRF family on the −89 CD68 composite element. A, labeled −89 CD68 probe and THP-1 nuclear extracts were used in supershift experiments with antisera against IRF family members and PU.1 as indicated. B, EMSA using the −89 CD68 probe mutated in the IRF half-site (−89mutIRF) incubated with THP-1 nuclear extracts with or without specific antisera as indicated. No complexes were observed with a labeled probe mutated in the PU.1 half-site with THP-1 nuclear extracts (data not shown). The asterisk denotes an uncharacterized complex.
lines Raji, Thiel, and A20, and a murine spleen extract. All extracts were normalized for protein content by BCA and 3-fold more monocytic cell extracts were loaded as compared with the B lymphocytes and spleen extracts. As shown in Fig. 7, IRF-4 and IRF-8 appear to be differentially expressed in the different cell lines tested. IRF-8 is predominantly expressed in THP-1, HL60, and U937 cells (right panel), whereas IRF-4 appears to be restricted to the B lymphocytic cell lines (left panel). Thiel and A20 cells express very little IRF-8, whereas Raji and spleen cells express levels almost equivalent to the U937 and HL60 cells.

We wanted to determine whether this differential expression of IRF-4 and IRF-8 was also true for primary human B cells and human monocytes. Nuclear extracts were prepared from B cells, which were positively selected from human buffy coats from different donors using CD19 cross-linked Dynal beads, and day one monocytes/macrophage cultures according under “Experimental Procedures.” IRF-4 is primarily expressed in the human B cells with significant levels of IRF-8 also detected (Fig. 7B). This expression level is very similar to levels observed in human and murine B cell extracts (Fig. 7A). Human monocytes, on the other hand, show a similar expression pattern to THP-1, HL60, and U937 cells and express low levels of IRF-8, whereas IRF-4 expression is undetectable. In conclusion, the expression of IRF-4 and IRF-8 in primary human B lymphocytes and human monocytes corresponds to the patterns of expression detected in the human cell lines used in these studies.

**IRF-4 Associates with the CD68 Promoter in B Cells**—We subsequently sought to determine whether IRF-4 binds the CD68 promoter in vivo in macrophages and/or B cells. THP-1 and U937 cells were cross-linked as before and protein-DNA complexes were immunoprecipitated with an anti-IRF-4 specific antibody. Buffer alone and IgG immunoprecipitates were prepared as PCR and antibody specificity controls, respectively. It has been reported that IRF-4 interacts with PU.1 at a composite element within an upstream enhancer of the human IL-1β gene (34) and a similar site within the proximal promoter.
of the human Toll-like receptor (TLR) 4 gene in vitro in EMSA experiments using myeloid cell nuclear extracts (32). We synthesized genomic primers directed to both of these promoter regions as positive controls for IRF-4 binding and to the human 12/15 lipoxygenase (12/15LO) gene as a negative control. We did not detect binding of the IRF-4 factor to the 12/15LO gene in any of the cell lines tested (Fig. 8, A and B). However, we reproducibly detected IRF-4 binding to each of the target gene promoters, IL-1β and TLR-4, in the monocytic cells, but we were unable to show association of IRF-4 with the CD68 proximal promoter in macrophages (Fig. 8A).

To determine whether IRF-4 is associated with the CD68 promoter in B lymphocytic cell lines we cross-linked Raji and Thiel cells and immunoprecipitated IRF-4-containing DNA complexes with an anti-IRF-4 antiserum. As a positive control for IRF-4 binding we synthesized primers flanking the PU.1/IRF-8 complexes can repress CD68 promoter activity in human mononuclear cells. Furthermore, chromatin immunoprecipitation experiments reveal that IRF-4 associates with the CD68 promoter in vivo in B lymphocytes and not monocytes.

Our results show that the Ets family transcription factors Elf-1, PU.1, and Fli-1 bind the CD68 proximal promoter in vitro and in vivo. To date, there are over 35 characterized members of the Ets transcription factor family reported to be involved in a vast number of processes from cell differentiation, proliferation, hematopoiesis, and apoptosis (36–38). They form a winged helix loop helix structure with the DNA and recognize a core (C/A)GGA(A/T) sequence with flanking sequences defining the precise binding specificity (39). PU.1 is the most divergent member and is required for the proper generation of myeloid and lymphoid lineages (40, 41). High levels of PU.1 expression promote macrophage differentiation and lower levels are associated with the development of B lymphocytes (42, 43). PU.1 regulates the proliferation and differentiation of these lineages by controlling the expression of receptors for macrophage colony stimulating factor (20) and interleukin 7 (IL-7Rα) (44). Elf-1 and Fli-1 are also expressed in hematopoietic cells and are key components of an enhanceosome complex that activates the stem cell leukemia gene (SCL), which is critical for the normal development of all hematopoietic lineages (45). Elf-1 has also been shown to be involved in the regulation of a number of T-cell specific genes including CD3ζ and CD4 (46, 47).

**DISCUSSION**

In this study we have investigated the transcriptional regulation of CD68 in human macrophages and defined the minimal proximal promoter that confers full reporter activity in these cells. We have identified two cis-regulatory elements that are critical for CD68 activity in macrophages that bind members of the Ets family of transcription factors including Elf-1, PU.1, and Fli-1. One of these elements is a composite site that binds PU.1 and interferon regulatory factor members, IRF-4 and IRF-8. Typically PU.1/IRF-4 and PU.1/IRF-8 heterocomplexes possess transcriptional activation activity in immune cells. In contrast, our results provide evidence that PU.1/IRF-4 and PU.1/IRF-8 complexes can repress CD68 promoter activity in human mononuclear cells. Furthermore, chromatin immunoprecipitation experiments reveal that IRF-4 associates with the CD68 promoter in vivo in B lymphocytes and not monocytes.

FIG. 8. IRF-4 associates with the CD68 proximal promoter in vivo in B cells. Formaldehyde cross-linked chromatin prepared from 1 × 10⁶ THP-1 and U937 (A) and Raji and Thiel (B) cells were incubated with antisera specific for IRF-4 or an IgG control as indicated. As a positive control an aliquot representing 0.03% of the total input chromatin (Total) was incubated in the PCR reactions. An additional control included a sample lacking chromatin (Mock). Immunoprecipitates and controls were analyzed by PCR using primers specific to the proximal promoters of the following genes: human interleukin-1β gene (IL-1β), human Toll-like receptor-4 gene (TLR-4), 12 lipoxygenase gene (12/15LO), the B-lineage specific CD20 gene, and human CD68. Results represent data obtained from n = 9 for Raji and THP-1 cells, and n = 3 for Thiel and U937 cells.

...
immunoprecipitation analysis confirmed that Elf-1 is bound to the proximal promoter in vivo in THP-1 cells (Fig. 4B). Western blot analysis of numerous monocytic cell lines including THP-1, HL60, MALU, P388.D1, and non-macrophage cell lines including COS-7, 293, NIH 3T3, CV-1, HeLa, and Chinese hamster ovary show abundant expression of Elf-1 protein (data not shown). We were unable to find any cell line that did not express Elf-1 to test relative promoter activity and we propose that the elevated levels of activity that we see with the CD68 promoter construct in non-macrophage cell lines is due to the presence of endogenous Elf-1. Also, the 4-fold induction that we observe when we overexpress Elf-1 in transient transfection assays is possibly an under-representation of the true activating potential of this Ets factor on the CD68 promoter.

Chromatin immunoprecipitation analysis, to investigate in vivo transcription factor binding, indicates that Fli-1 binds the CD68 proximal promoter with higher affinity as compared with the 3′-UTR region (Fig. 4C). There are no consensus Ets binding sites within this 3′-UTR region and as such it is possible to speculate that Fli-1 binds directly to an uncharacterized consensus sequence or indirectly by associating with a factor bound to the DNA. It does, however, appear to associate with the −106 site within the proximal promoter in EMSA (Fig. 3A) and transactivates the CD68 promoter −6-fold in transient transfection assays (Fig. 4A). We have shown that Elf-1 preferentially binds this site and as such it is possible to speculate that Fli-1 orchestrates enhancer activity by competing with Elf-1 for the −106 site. If this is indeed the case, this redundancy may represent an essential mechanism to ensure that CD68 expression is possible in all macrophage subpopulations in vivo. Fli-1 association with the CD68 promoter may also represent a subpopulation of THP-1 cells in a fixed stage of the cell cycle or a particular activation state. Wells et al. (48) showed that several different E2F factors associate with the dihydrofolate reductase promoter at a given time during the cell cycle (49). They propose a stochastic model whereby multiple factors can compete for binding to a particular site, but the actual complex formed is dependent on the availability and activation states of the individual transcription factors within the nucleus.

It is becoming increasingly evident that Ets factors can modulate transcription by forming complexes with members of other transcription factor families including AP-1 (50, 51), NF-κB (52, 53), and IRF (54–56). Many of these transcription factors have DNA binding sites adjacent to the Ets factor binding site. Our results show that the second cis-regulatory element located immediately 3′ to the Elf-1 binding site is a composite element that binds PU.1 and interferon regulatory factor family members IRF-4 and IRF-8. Similar sites have been characterized within the Igκ (55), Igλ (57), and human IL-1β enhancers (34), CD20 (35), gp91phox (58), and human TLR-4 promoters (32). There are 10 IRF family members characterized to date and up to 6 viral homologues but only IRF-4 and IRF-8 have been shown to physically associate with PU.1 (59–61). Once PU.1 binds to the DNA it recruits either IRF-4 or IRF-8 to the PU.1/IRF composite element via a proline-glutamine-serine-threonine-rich (PEST) domain of PU.1 (62). Knockout studies in mice have revealed that IRF-4 is primarily restricted to lymphoid cells and is responsible for mature B and T cell function, whereas IRF-8 is predominately expressed in myeloid cells and essential in promoting the commitment of these progenitor cells to the macrophage lineage (63–65).

Both IRF-4 and IRF-8 typically function as transcription activators when they form heterocomplexes with PU.1. However, our data demonstrate that IRF-4 and IRF-8 can repress CD68 activity in a dose-dependent manner in THP-1 cells (Fig. 6, B and C). Transcriptional repression requires DNA contact as mutations that abolish IRF binding, as assessed by EMSA (Fig. 5A), reversed this repressive effect. Antibody supershift experiments with anti-PU.1 antisera also confirmed that neither IRF-4 nor IRF-8 could bind the composite element in the absence of association with PU.1 (Fig. 5A). Also, IRF-8 failed to repress CD68 promoter activity when the PU.1 half-site was mutated (Fig. 6C). Intriguing, IRF-4 was still able to repress activity by as much as 60% when the PU.1 site was altered (Fig. 6B). This may reflect differences in binding affinities between IRF-4 and IRF-8 for the DNA in the presence of endogenous PU.1 or it is possible that IRF-4-dependent repression is mediated through interactions with Elf-1 at the −106 site. EMSA analysis revealed that PU.1 can also bind the −106 site (Fig. 3A, A and B) and as such it is likely that in the absence of the −89 PU.1 site, PU.1 may compete with Elf-1 for the −106 site and recruit IRF-4.

One interesting observation with the PU.1/IRF mutants and their effects on CD68 promoter activity was that mutations of the IRF half-site failed to have any effect on promoter activity in THP-1 cells, whereas mutation of the PU.1 binding site reduced activity by as much as 75% (Fig. 6A). This would suggest that IRF factors are not necessary for CD68 promoter activity in myeloid cells but that Ets factor binding is critical. Western blot data and chromatin immunoprecipitation analysis enabled us to further understand both the reason why the −89mIRF CD68 promoter construct maintained full activity in THP-1 cells and more importantly, the significance of the PU.1/IRF-4 and PU.1/IRF-8 mediated repression of CD68 activity in these cells. Our data show that IRF-8 is predominately expressed in myeloid cell lines including primary monocytic cells from two separate donors, whereas IRF-4 is poorly expressed in these cells but abundantly expressed in B cell lines including Raji, Thiel, A20, murine spleen extracts, and primary B lymphocytes (Fig. 7, A and B). B cells do not express CD68, which raises the possibility that the function of IRF-4 or IRF-8 may be to repress CD68 activity in non-macrophage cell lines. Chromatin immunoprecipitation data confirmed our hypothesis and showed that IRF-4 was indeed bound to the CD68 promoter in vivo in the two B cell lines tested, Raji and Thiel, and was not found to associate with this promoter in THP-1 or U937 cells (Fig. 8, A and B). It was previously demonstrated that IRF-4 modulated CD20 in a lineage and stage-specific manner (35). CD20 was maximally expressed in pre-B cells and declined as cells differentiated to plasma cells. This was dependent on interactions with IRF-4 and PU.1 at the composite site, whereby PU.1 was the limiting factor. Further experiments are in progress to determine whether this association of IRF-4 to the CD68 promoter in B cells is an indication that an analogous mechanism may exist for CD68. Because IRF-4 is predominantly restricted to lymphoid cells it would be a limiting factor as hematopoietic cells differentiate into myeloid cells, which would favor CD68 expression in these cell types. Based on these data we propose that IRF-4 interacts with PU.1 and binds the composite element within the CD68 promoter in vivo in B lymphocytes to specifically down-regulate CD68 expression in these cells. It is also worth noting that the positive controls in our chromatin immunoprecipitation experiments (Fig. 8, A and B) show for the first time that IRF-4 is associated in vivo with CD20, human IL-1β, and human TLR-4 promoters.

We propose that IRF-4 plays an important role in down-regulating CD68 expression in B lymphocytes. Our studies have identified a new regulatory motif within the CD68 promoter and extended our understanding of the molecular mechanisms that direct macrophage-restricted expression of the human CD68 gene.
Multiple Ets Factors and Interferon Regulatory Factor-4 Modulate CD68 Expression in a Cell Type-specific Manner
Dawn O'Reilly, Carmel M. Quinn, Tariq El-Shanawany, Siamon Gordon and David R. Greaves

J. Biol. Chem. 2003, 278:21909-21919.
doi: 10.1074/jbc.M212150200 originally published online April 3, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M212150200

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 64 references, 41 of which can be accessed free at http://www.jbc.org/content/278/24/21909.full.html#ref-list-1