

Mechanisms of regulation of the MacMARCKS gene in macrophages by bacterial lipopolysaccharide

Sandy Chang,* Katryn J. Stacey, Jianmin Chen,* Elaine O. Costelloe, Alan Aderem,* and David A. Hume

*Departments of Biochemistry and Microbiology and Centre for Molecular and Cellular Biology, University of Queensland, Australia; and *Department of Immunology, University of Washington, Seattle*

Abstract: Bacterial lipopolysaccharide (LPS) stably induced the protein kinase C substrate, MacMARCKS, in murine resident peritoneal macrophages; initial induction of MacMARCKS mRNA was detected within 15 min and was protein synthesis-independent. This response was observed in the macrophage cell line RAW264, and occurred also in response to plasmid DNA, a partial mimetic of other responses to LPS. In murine bone marrow-derived macrophages, MacMARCKS was expressed constitutively due to induction by macrophage colony-stimulating factor. Nuclear run-on transcription revealed that, like tumor necrosis factor α (TNF- α), MacMARCKS was transcribed constitutively in RAW264 cells. The MacMARCKS promoter was sequenced to -1.7 kb and the transcription start site determined. Transient transfections of RAW264 cells revealed that the 113-bp GC-rich proximal promoter contained all the elements required for both high basal activity and 15- to 20-fold activation by LPS. *J. Leukoc. Biol.* 66: 528–534; 1999.

Key Words: endotoxin · transcription · transfection · Sp1

INTRODUCTION

The major alanine-rich C kinase substrate (MARCKS) is a substrate of protein kinase C that binds calmodulin and components of the actin cytoskeleton, and becomes phosphorylated in a wide range of processes including mitogenesis, neurosecretion, and macrophage activation [1, 2]. MacMARCKS (also known as MARCKS-related protein and F52) was identified as a major lipopolysaccharide (LPS)-inducible protein in macrophages [3]. It appears to be involved in phagocytosis in association with components of the cytoskeleton in cells of this lineage [4, 5] and possibly in integrin-mediated cell adhesion and spreading [6]. MacMARCKS is also expressed in cells of the central nervous system, where its subcellular localization implies a function in neurosecretion. Consistent with a role in cells of this lineage, and its pattern of expression in the developing mouse embryo and differentiating PC12 cells [7], mice with introduced null mutations in this gene have been shown to be anencephalic due to a failure of neural tube closure [8, 9]. Despite the close association of Mac-

MARCKS with phagocytic vesicles, macrophages derived from such animals are not deficient in endocytosis [10].

Studies of the transcriptional control of the MacMARCKS gene have indicated that the entire gene, including 407 bp of proximal promoter, is able to drive high-level expression of the gene product when transfected into fibroblasts that lack endogenous expression of the gene [11]. In this study we have examined the mechanisms that control the level of expression of MacMARCKS mRNA and protein in macrophages.

MATERIALS AND METHODS

RAW264 macrophages were obtained from the American Type Culture Collection and maintained in bacteriological Petri dishes as described [12, 13]. Peritoneal macrophages from female ICR mice were obtained by two rounds of lavage using ice-cold sterile phosphate-buffered saline. Bone marrow-derived macrophages were obtained by cultivation of femoral bone marrow cells for 7 days in recombinant macrophage colony-stimulating factor (CSF-1, a gift from Chiron) as described [12, 13]. Details of experimental treatments are in the legends to Figures 1–6. Polyclonal antiserum against murine MacMARCKS was prepared by injecting rabbits with a purified GST-MacMARCKS fusion protein. Antibody was affinity purified from sera using strips of polyvinylidene difluoride membrane with recombinant MacMARCKS bound. Detection of MacMARCKS protein takes advantage of the fact that it is co-translationally myristoylated. The procedure and detection of MacMARCKS in cells prelabeled with [3 H]myristic acid has been described by Allen and Aderem [14].

For transfection analyses, a 17-kb genomic DNA clone containing the entire murine MacMARCKS gene was isolated from a 129sv genomic library using a MacMARCKS 5' cDNA probe. A 1.7-kb NheI fragment was completely sequenced and was found to cover the 5' end of the gene, including the transcription start site (see below). This fragment was subcloned into the luciferase reporter plasmid, pGL2 (Promega) and into pBluescript. 5' Nested truncations of the promoter were generated by Exonuclease III cleavage using the Erase-a-Base method (Promega) in pBluescript and the resulting subclones were also transferred into pGL2. Transfections in the murine RAW264 cell line were carried out by electroporation as described elsewhere and luciferase activity was determined after 18–24 h [13, 15]. Activity expressed as relative light units was normalized to protein measured with the use of a Bradford assay.

Levels of MacMARCKS mRNA were determined by either RNase protection or Northern analysis as described [12], depending on the availability of RNA. For RNase protection, a 315-base 32 P-labeled probe was synthesized corresponding to MacMARCKS coding sequence +485 to +745. The RNase protection

Correspondence: Professor David A. Hume, Department of Microbiology, University of Queensland, Q4072, Australia. E-mail D.Hume@cmcb.uq.edu.au

Present address of Sandy Chang: Department of Pathology, Brigham & Woman's Hospital, Harvard Medical School, Boston, MA 02115.

Received December 11, 1998; revised March 15, 1999; accepted March 16, 1999.

assay was carried out on 5 µg of total RNA with an RPAII kit (AMBION), with a 250-bp β-actin probe as an internal control. For mapping of the transcription start site of the gene by RNase protection, a 620-bp Xba1-Sac1 fragment of the gene covering the region from -413 to +203 was cloned into pBluescript and an RNA probe was generated with T3 polymerase. RNase protection was performed using 20 µg of RNA from LPS-stimulated RAW264 cells as template. The transcription start site was confirmed using primer extension with a 30-bp oligonucleotide complementary to the 5' end of the MacMARCKS cDNA (+174 to +203). The oligonucleotide was ³²P-labeled at the 5' terminus and mixed with 50 µg of RNA from LPS-stimulated RAW264 cells. After hybridization in buffer A from the Ambion RPAII kit, the primer was extended with Moloney murine leukemia virus reverse transcriptase (Boehringer-Mannheim) in the presence of 50 µg/mL actinomycin D. The product was resolved in parallel with a dideoxy sequencing ladder on 6% polyacrylamide/7 M urea.

RESULTS

Previous studies provided some indication that level of MacMARCKS mRNA was inducible by LPS in murine peritoneal macrophages [3]. **Figure 1A** examines the LPS dose dependence of this induction. In these cells, which are relatively inactive in terms of expression of other LPS-responsive genes and are known to be poorly endocytic, MacMARCKS expression was variable in different experiments, but always low. Addition of as little as 0.1 ng/mL LPS produced a substantial induction of the level of MacMARCKS mRNA and 1 ng/mL LPS provided near-maximal induction after 4 h of treatment. The ability of LPS to induce MacMARCKS mRNA was paralleled by a substantial increase in the level of protein (Fig. 1A) as described previously.

The kinetics of induction of MacMARCKS mRNA levels in peritoneal macrophages are examined in Figure 1B. A detectable increase in MacMARCKS mRNA was observed only 5 min after addition of a maximal dose of LPS, but the expression continued to increase for up to 2 h. Not surprisingly, the level of MacMARCKS protein, detected by immunoprecipitation from cells labeled with [³H]myristic acid, increased more slowly but was still detectable after only 20 min. The induction of MacMARCKS mRNA by LPS was so rapid that a requirement for intervening protein synthesis appeared inherently unlikely. In keeping with this view, MacMARCKS mRNA levels, like those of TNF-α, were actually found to be induced by treatment with the protein synthesis inhibitor cycloheximide, and super-induced on subsequent addition of LPS (data not shown).

Peritoneal macrophages are not available in sufficient numbers for more extensive mechanistic studies and cannot be transfected. For this reason, we examined the LPS-responsive murine macrophage cell line RAW264 and bone marrow-derived macrophages (BMDM) cultivated from marrow in the presence of macrophage CSF-1. In RAW264 cells, MacMARCKS was expressed constitutively at low levels, but could be very greatly induced by addition of 100 ng/mL LPS (**Fig. 2**). Like TNF-α, which was examined in parallel, MacMARCKS was maximally induced after 1 h, but the peak of expression was sustained for somewhat longer, being still maximal after 8 h (Fig. 2). Dose-response curves for induction of the two genes were indistinguishable (data not shown). Hence, RAW264 cells can be used as a model to understand LPS regulation of the MacMARCKS gene. We have shown recently that some actions of LPS on macrophage gene expression are mimicked by

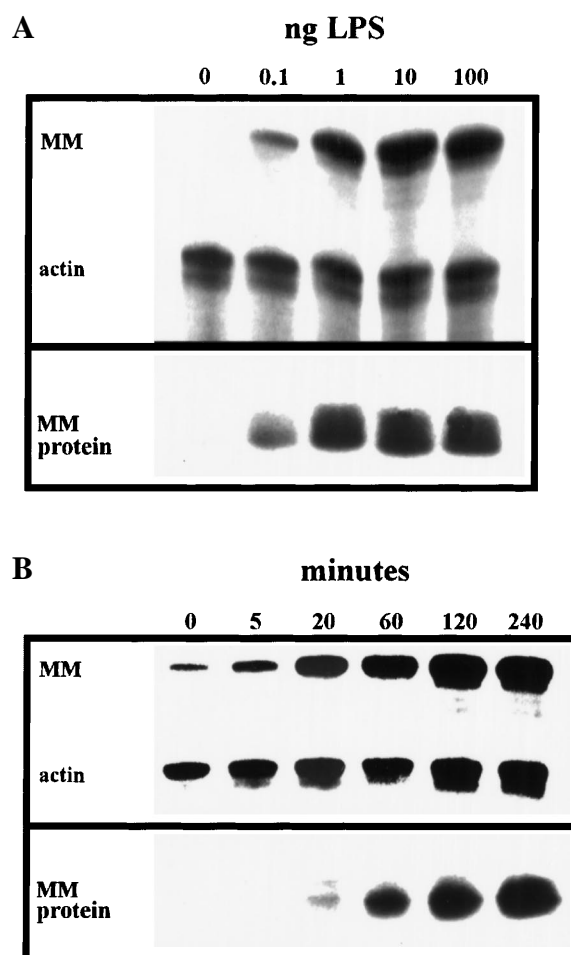


Fig. 1. Induction of MacMARCKS mRNA in peritoneal macrophages. (A) Dose-response analysis of LPS induction of MacMARCKS mRNA and protein. Top, peritoneal macrophages were stimulated with LPS as indicated for 4 h and total RNA was isolated and analyzed by RNase protection (Materials and Methods). The upper band is the 315-bp MacMarcks (MM) protected fragment. Bottom, peritoneal macrophages were labeled with [³H]myristic acid and stimulated with LPS as above. MacMARCKS was immunoprecipitated from the cell lysate with anti-MacMARCKS antibody, resolved on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and detected by fluorography. (B) Time course of induction of MacMARCKS mRNA and protein. Peritoneal macrophages were incubated with LPS (100 ng/mL) for the times indicated and MacMARCKS mRNA and protein were detected as described for panel A.

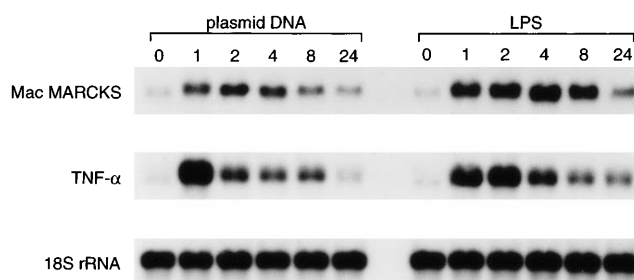


Fig. 2. Induction of MacMARCKS mRNA in RAW264 cells by LPS or plasmid DNA. RAW264 cells were incubated for the times indicated with either LPS (100 ng/mL) or pBluescript plasmid DNA (2 µg/mL). Northern blots prepared from total RNA were probed for expression of MacMARCKS and TNF-α and loading is controlled by probing with an 18S RNA oligonucleotide.

plasmid DNA, which is recognized by virtue of the presence of unmethylated CpG motifs [16, 17]. Recognition of the effect of plasmid DNA is important because it is a constraint upon the interpretation of transient transfections. Addition of plasmid DNA to RAW264 cells at concentrations used in transfections was also able to induce MacMARCKS mRNA. The response was approximately half as great as with LPS and was less sustained (Fig. 2). As observed previously with other LPS-responsive genes such as TNF- α , the response to plasmid DNA was abolished by treatment with DNase1, eliminating any possible role for contaminating LPS in the plasmid preparation (data not shown).

In contrast to peritoneal macrophages and RAW264, in BMDM MacMARCKS mRNA was found to be expressed constitutively at high levels. The addition of either LPS or plasmid DNA did not further elevate the level of mRNA (data not shown). In the same studies, and many other studies in our laboratory, LPS-responsive genes such as TNF- α and plasminogen activator-inhibitor type 2 are undetectable in BMDM in the absence of added LPS, so the constitutive expression of MacMARCKS cannot be due to LPS contamination [see refs. 16, 17]. We showed previously that CSF-1 regulates the response of BMDM to the protein kinase C agonist, phorbol myristate acetate (PMA) in terms of induction of urokinase plasminogen activator [12, 13]. Hence, we examined the expression of MacMARCKS mRNA in BMDM starved of CSF-1 and then refed. **Figure 3** shows that MacMARCKS mRNA was reduced to undetectable levels in starved cells and was induced by re-addition of CSF-1, regaining the steady state elevated level after 8 h. The subsequent decline observed in Figure 3 is due to CSF-1 depletion and can be prevented by addition of higher relative concentrations. MacMARCKS mRNA appears to be maintained for as long as CSF-1 is present (data not shown). In contrast to the rapid induction by LPS in responsive cells, the time course of CSF-1 induction of MacMARCKS was slow and resembled that of induction of urokinase plasminogen activator under the same conditions [see ref. 12], whereas a typical early response gene, the transcription factor Ets-2 [12, 13], was induced more rapidly and transiently.

To examine the mechanism of induction of MacMARCKS by

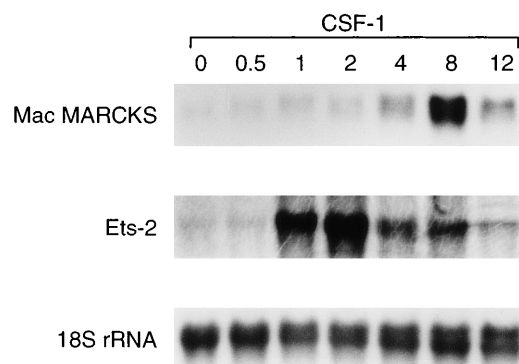


Fig. 3. Induction of MacMARCKS mRNA in bone marrow-derived macrophages by macrophage CSF-1. Murine bone marrow-derived macrophages were starved of CSF-1 overnight, then recombinant human CSF-1 (5000 U/mL) was added. Total RNA was isolated at the times indicated and Northern blots were prepared and probed for expression of MacMARCKS and Ets-2 as indicated. 18S RNA is used as a loading control.

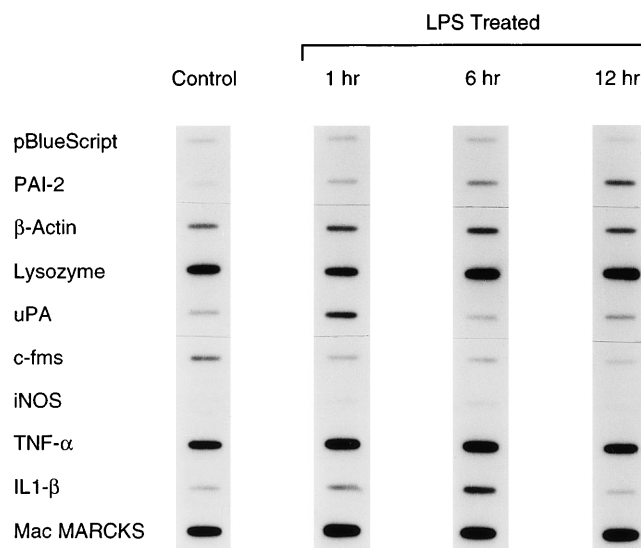


Fig. 4. Transcriptional regulation of LPS-responsive genes in RAW264 cells. RAW264 cells were incubated for the times indicated with 100 ng/mL LPS, nuclei were isolated, and labeled run-on transcripts were prepared (Materials and Methods). Plasmid DNAs containing full-length murine cDNAs encoding each of the genes of interest as indicated were slot-blotted onto the membrane, which was then hybridized with the labeled probes, washed, and exposed to autoradiography.

LPS, we performed nuclear run-on transcription assays using RAW264 cells. As a positive control for genes that are known to be induced by LPS we used interleukin-1 β (IL-1 β) and plasminogen activator inhibitor type 2 (PAI-2) and, conversely, we used *c-fms* as a control gene that is transcriptionally repressed by LPS. We also examined TNF- α , which is reported to be regulated both transcriptionally and posttranscriptionally. The results are shown in **Figure 4**. Both MacMARCKS and TNF- α were transcribed constitutively at high levels in RAW264 cell nuclei in the absence of LPS, and addition of LPS produced only a marginal further increase in transcription of either of these genes. In contrast, transcription of IL-1 β PAI-2 was clearly induced from a very low basal level by addition of LPS over an extended time course. Urokinase plasminogen activator mRNA is induced transiently by LPS [unpublished results] and, in accord with this pattern, transcription was induced after 1 h but returned to baseline thereafter. Induction of iNOS was also induced after 6 h, although the level is very low and difficult to demonstrate photographically. Conversely, transcription of *c-fms*, which was already low by comparison to MacMARCKS and TNF- α , was repressed still further. All of these controls demonstrate that there is a clear response to LPS under the conditions of the experiment, but both MacMARCKS and TNF- α are transcribed constitutively and neither is markedly induced transcriptionally by LPS in RAW264 cells.

The results from the run-on transcription assay would suggest that the MacMARCKS promoter would be constitutively active in RAW264 cells. The 5' flanking sequence was determined as indicated in **Figure 5**. This extends the previously reported sequence [11] by 1.4 kb. The presumptive proximal promoter is very GC rich, somewhat reminiscent of a house-keeping gene, and has two possible AT-rich TATA like elements. The human MacMARCKS promoter has also been

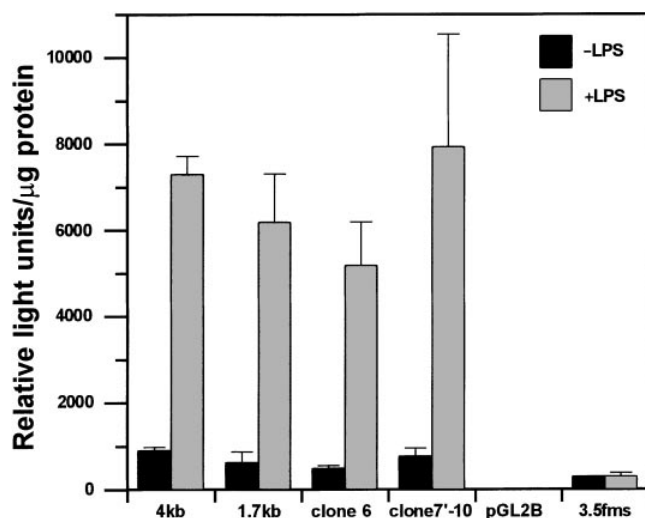


Fig. 6. Transient transfection analysis of the effect of LPS on activity of MacMARCKS promoter-luciferase reporter genes in RAW264 cells. RAW264 cells were transiently transfected by electroporation with the MacMARCKS promoter-luciferase reporter constructs as indicated. Clone 6 contains 267 bp and p7'-10 luc 113 bp of MacMARCKS promoter, respectively, while pGL-B is the parent promoterless vector. The 3.5-kb murine *c-fms* promoter is a macrophage-specific promoter that is known not to be LPS-responsive. Twenty-four hours posttransfection cells were harvested and luciferase activity was determined as described in Materials and Methods. Results are the average plus or minus standard deviation of triplicate determinations. The experiment is representative of three separate experiments.

construct, including an initial 2.3 kb of unsequenced 5' flanking region, was approximately twofold more active but no more LPS-responsive. By comparison, endotoxin-responsive HIV-1-LTR assayed under comparable conditions generally gives less than fivefold *trans*-activation in response to LPS, in part because of the effect of the reporter plasmid DNA itself on the baseline activity [16, 17]. Sequential deletion down to the proximal 113 bp alone caused almost no diminution of either basal or LPS-stimulated activity. This most proximal deletion eliminated a perfect binding site at -120 (GAGAGGAA) for the macrophage-specific Ets family transcription factor PU.1 as well as several other candidate upstream regulatory elements. The 113-bp proximal promoter region is remarkably GC-rich and contains at least four copies of the perfect consensus binding site for the basal transcription factor Sp1 as well as numerous other GC-rich elements that probably also bind this factor. The lack of induction of the murine *c-fms* promoter (Fig. 6), which lacks Sp1 sites [19], demonstrates that the response to LPS is promoter-specific. In other experiments, minimal promoters such as that of the HIV-1-LTR, SV40 and urokinase plasminogen activator, that contain Sp1 sites but have much lower basal activity and are less GC-rich, gave a two- to fourfold activation in response to LPS under comparable conditions (data not shown).

DISCUSSION

MacMARCKS was identified in macrophages because of its putative involvement in the activation by LPS of the response of these cells to subsequent stimulation by the protein kinase C

agonist, PMA [3]. In this study we have shown that very low concentrations of LPS induces MacMARCKS mRNA within minutes in macrophages. Like a growing list of LPS-inducible genes, MacMARCKS was also inducible by plasmid DNA (Fig. 2). The ability of plasmid DNA, via the presence of immunostimulatory DNA sequences containing unmethylated CpG, to partly mimic the response to LPS has been implicated in the efficacy of plasmid DNA vaccines [see ref. 16].

The data obtained with BMDM clearly distinguish MacMARCKS from other LPS-inducible genes. In contrast to MacMARCKS, TNF- α is undetectable in BMDM in the presence or absence of CSF-1, but is massively induced by either LPS or plasmid DNA [16]. MacMARCKS was inducible by CSF-1 in BMDM (Fig. 3). The failure of these cells to respond to further stimulation implies that the responses to LPS and CSF-1 are not additive. The induction of MacMARCKS by CSF-1 may contribute to the synergistic interactions between CSF-1 and PMA measured in terms of induction of urokinase plasminogen activator mRNA [12, 13]. Apart from sensitizing cells to PMA, CSF-1 and LPS share the ability to induce profound extensive spreading of macrophages on a substratum. Induction of MacMARCKS probably functions in regulation of cell adhesion, spreading, membrane trafficking, and endocytosis [1], despite evidence from the knockout mouse that this function is partly redundant [10].

Nuclear run-on transcription assays in RAW264 cells showed that MacMARCKS was transcribed constitutively at a high rate in RAW264 macrophages and the production of transcripts as detected by the run-on transcription assay was no more LPS-inducible than transcription of β -actin, which is not LPS-inducible at the level of mRNA expression (data not shown). The finding with TNF- α is completely different from recent findings of others, in which fivefold transcriptional activation was observed 2 h after LPS treatment of RAW264 cells [20]. The published experiments did not include positive controls of other LPS-inducible or repressible genes and the time chosen is actually well after the major peak of TNF- α mRNA expression. Our data support the view that TNF- α is regulated primarily at a posttranscriptional level [21]. The mechanisms involved in TNF- α and MacMARCKS regulation are not universal in LPS responses; many LPS-responsive genes, including IL-1 β , PAI-2, and iNOS as shown in Figure 4 are activated primarily at the level of transcription initiation.

In part, the run-on transcription assay was consistent with the transient transfections. The MacMARCKS promoter is constitutively highly active in RAW264 cells, more so than the macrophage-specific *c-fms* promoter, and the activity was retained in a deletion containing only the 113-bp GC-rich proximal promoter (Fig. 6), which contains multiple consensus Sp1 binding sites (Fig. 5). Although Sp1 is often regarded as a ubiquitous transcription factor governing the expression of housekeeping genes, in a previous study of the proximal promoter of the HIV-1-LTR we observed LPS induction of nuclear proteins that bound the SP1 consensus sequence sequences and were recognized by anti-Sp1 antibodies [17]. Others have also reported on activation of Sp1 by LPS [22, 23] and multiple Sp1 sites are found in the proximal promoters of many genes that are myeloid-specific and/or related to LPS

actions, including CD14, CD11b, mannose receptor, and the TNF- α genes [23–29]. In many of the promoters that are constitutively active in macrophages [e.g., 28, 29], Sp1 has been found to cooperate with the macrophage-specific transcription factor PU.1 bound to adjacent motifs. In transient transfections, elimination of the perfect PU.1 box motif at -120 did not reduce basal or LPS-stimulated MacMARCKS promoter activity in RAW264 cells but it seems likely that this site contributes to myeloid-specific expression in the endogenous chromatin context. Given the multiplicity of GC-rich elements in the MacMARCKS promoter, another LPS-inducible protein, EGR-1 [26, 30], probably also contributes to LPS-inducible activity in transient transfections. GC-rich proximal promoters are very widespread among genes expressed in other cell types. In general, multiple Sp1 binding sites are functionally redundant, with deletion of individual sites causing small reductions in activity in transient transfection [31, 32]. A recent study of the mouse *aprt* gene suggests that some apparently redundant Sp1 elements contribute to protection of the gene against methylation-dependent inactivation, a function that could only be assessed in transgenes or stable transfections.

The obvious question that arises from our study is why the transcriptional activation that is apparent in transient transfections despite the possible contribution of plasmid DNA to the basal promoter activity was not observed in run-on transcription assays. One possibility is that Sp1-dependent induction of MacMARCKS is masked by parallel induction of a wide range of other cellular genes (including the β -actin gene used as a control) that also contain Sp1 sites. Alternatively, transient transfection by electroporation introduces a very large number of DNA templates into cells. The presence of so many Sp1 binding sites in the MacMARCKS promoter may titrate the endogenous pool of nuclear proteins so that it falls into a range where the induction by LPS causes a more marked increase in activity of the transfected gene(s). Finally, in the case of LPS induction of TNF- α in a murine macrophage cell line, there is evidence that LPS acts to increase transcription elongation [33]. In the run-on transcription assay we have not distinguished initiation and elongation, having used a full-length cDNA as probe, so it remains possible that elongation is also a site of regulation of MacMARCKS. In principle, transcription elongation may be generated by DNA structures or proteins bound at the point of premature termination or by alterations in the intrinsic processivity of RNA polymerase molecules generated at the site of initiation. Because events occurring on the promoter influence the ability of RNA polymerase to complete a mature luciferase transcript, and therefore to make a luciferase protein, transient transfections actually measure both initiation and elongation. Yankulov et al. [34] have provided evidence in model systems that RNA polymerase II processivity is controlled by transcription factors bound to the proximal promoter and can be as important as the stimulation of initiation in determining the expression of a reporter gene. Hence, the reporter gene analysis and run-on transcription assays are not necessarily incompatible.

The addition of LPS to macrophages generates a cascade of different signaling pathways that appear to involve all the known signaling molecules in different combinations in differ-

ent macrophage populations [35]. This complexity is compounded by the fact that endogenous cytokines induced in response to LPS rapidly initiate autocrine loops that may amplify, terminate, or ramify the primary signal [35]. No two genes that respond to LPS in macrophages are regulated by precisely the same signaling pathway, and the data obtained with MacMARCKS indicate that it is also regulated in a gene-specific manner. Although this complexity is daunting, it offers the hope that it will be possible to dissect LPS action to eventually separate those aspects that are responsible for endotoxin toxicity.

ACKNOWLEDGMENTS

This work was supported by grants from the National Health and Medical Research Council of Australia and Grants AI25032 and AI32972 from the National Institutes of Health, Bethesda, MD. The Centre for Molecular and Cellular Biology is a Special Research Centre of the Australian Research Council.

REFERENCES

1. Aderem, A. (1995) The MARCKS family of protein kinase-C substrates. *Biochem. Soc. Trans.* 23, 587–591.
2. Blackshear, P. J. (1993) The MARCKS family of cellular protein kinase C substrates. *J. Biol. Chem.* 268, 1501–1504.
3. Li, J., Aderem, A. (1992) MacMARCKS, a novel member of the MARCKS family of protein kinase C substrates. *Cell* 70, 791–801.
4. Allen, L. A., Aderem, A. (1996) Molecular definition of distinct cytoskeletal structures involved in complement- and Fc receptor-mediated phagocytosis in macrophages. *J. Exp. Med.* 184, 627–637.
5. Allen, L. A., Aderem, A. (1996) Mechanisms of phagocytosis. *Curr. Opin. Immunol.* 8, 36–40.
6. Li, J., Zhu, Z., Bao, Z. (1996) Role of MacMARCKS in integrin-dependent macrophage spreading and tyrosine phosphorylation of paxillin. *J. Biol. Chem.* 271, 12985–12990.
7. Chang, S., Hemmings, H. J., Aderem, A. (1996) Stimulus-dependent phosphorylation of MacMARCKS, a protein kinase C substrate, in nerve termini and PC12 cells. *J. Biol. Chem.* 271, 1174–1178.
8. Chen, J., Chang, S., Duncan, S. A., Okano, H. J., Fishell, G., Aderem, A. (1996) Disruption of the MacMARCKS gene prevents cranial neural tube closure and results in anencephaly. *Proc. Natl. Acad. Sci. USA* 93, 6275–6279.
9. Wu, M., Chen, D. F., Sasakoa, T., Tonegawa, S. (1996) Neural tube defects and abnormal development in F52-deficient mice. *Proc. Natl. Acad. Sci. USA* 93, 2110–2115.
10. Underhill, D. M., Chen, J., Allen, L. A., Aderem, A. (1998) MacMARCKS is not essential for phagocytosis in macrophages. *J. Biol. Chem.* 273, 33619–33623.
11. Lobach, D. F., Rochelle, J. M., Watson, M. L., Seldin, M. F., Blackshear, P. J. (1993) Nucleotide sequence, expression, and chromosomal mapping of Mrp and mapping of five related sequences. *Genomics* 17, 194–204.
12. Stacey, K. J., Fowles, L. F., Colman, M. S., Ostrowski, M. C., Hume, D. A. (1995) Regulation of urokinase-type plasminogen activator gene transcription by macrophage colony-stimulating factor. *Mol. Cell Biol.* 15, 3430–3441.
13. Fowles, L. F., Martin, M. L., Nelsen, L., Stacey, K. J., Redd, D., Clark, Y. M., Nagamine, Y., McMahon, M., Hume, D. A., Ostrowski, M. C. (1998) Persistent activation of MAP kinases p42/44 and Ets-2 phosphorylation in response to CSF-1/*c-fms* signalling. *Mol. Cell Biol.* 18, 5148–5156.
14. Allen, L. A., Aderem, A. (1995) Protein kinase C regulates MARCKS cycling between the plasma membrane and lysosomes in fibroblasts. *EMBO J.* 14, 1109–1120.
15. Cassady, A. I., Stacey, K. J., Nimmo, K. A., Murphy, K. M., von der Ahe, D., Pearson, D., Botteri, F. M., Nagamine, Y., Hume, D. A. (1991) Constitutive expression of the urokinase plasminogen activator gene in murine RAW264 macrophages involves distal and 5' non-coding sequences that are conserved between mouse and pig. *Nucleic Acids Res.* 19, 6839–6847.

16. Stacey, K. J., Sweet, M. J., Hume, D. A. (1996) Macrophages ingest and are activated by bacterial DNA. *J. Immunol.* 157, 2116–2122.
17. Sweet, M. J., Stacey, K. J., Ross, I. L., Ostrowski, M. C., Hume, D. A. (1998) Involvement of Ets, rel and Sp1-like proteins in lipopolysaccharide-mediated activation of the HIV-1 LTR in macrophages. *J. Inflammation* 48, 67–83.
18. Stumpo, D. J., Eddy, R. L., Jr., Haley, L. L., Sait, S., Shows, T. B., Lai, W. S., Young, W. S., 3rd, Speer, M. C., Dehejia, A., Polymeropoulos, M., Blackshear, P. J. (1998) Promoter sequence, expression, and fine chromosomal mapping of the human gene (MLP) encoding the MARCKS-like protein: identification of neighboring and linked polymorphic loci for MLP and MACS and use in the evaluation of human neural tube defects. *Genomics* 49, 253–264.
19. Reddy, M. A., Yang, B.-S., Yue, X., Barnett, C. J. K., Ross, I. L., Sweet, M. J., Hume, D. A., Ostrowski, M. C. (1994) Opposing actions of c-ets/PU.1 and c-myc protooncogene products in regulating the macrophage-specific promoters of the human and mouse colony-stimulating factor-1 receptor (c-fms) genes. *J. Exp. Med.* 180, 2309–2319.
20. Raabe, T., Bukrinsky, M., Currie, R. A. (1998) Relative contribution of transcription and translation to the induction of tumor necrosis factor- α by lipopolysaccharide. *J. Biol. Chem.* 273, 974–980.
21. Lewis, T., Gueydan, C., Huez, G., Toulme, J. J., Kruys, V. (1998) Mapping of a minimal AU-rich sequence required for lipopolysaccharide-induced binding of a 55-kDa protein on tumor necrosis factor- α mRNA. *J. Biol. Chem.* 273, 13781–13786.
22. Jarvis, B. W., Qureshi, N. (1997) Inhibition of lipopolysaccharide-induced transcription factor Sp1 binding by spectrally pure diphosphoryl lipid A from *Rhodobacter sphaeroides*, protein kinase inhibitor H-8, and dexamethasone. *Infect. Immun.* 65, 1640–1643.
23. Oeth, P., Parry, G. C., Mackman, N. (1997) Regulation of the tissue factor gene in human monocytic cells. Role of AP-1, NF- κ B/Rel, and Sp1 proteins in uninduced and lipopolysaccharide-induced expression. *Arterioscler. Thromb. Vasc. Biol.* 17, 365–374.
24. Berrier, A., Siu, G., Calame, K. (1998) Transcription of a minimal promoter from the NF-IL6 gene is regulated by CREB/ATF and SP1 proteins in U937 promonocytic cells. *J. Immunol.* 161, 2267–2275.
25. Lopez-Rodriguez, C., Botella, L., Corbi, A. L. (1997) CCAAT-enhancer-binding proteins (C/EBP) regulate the tissue specific activity of the CD11c integrin gene promoter through functional interactions with Sp1 proteins. *J. Biol. Chem.* 272, 29120–29126.
26. Yao, J., Mackman, N., Edgington, T. S., Fan, S. T. (1997) Lipopolysaccharide induction of the tumor necrosis factor- α promoter in human monocytic cells. Regulation by Egr-1, c-Jun, and NF- κ B transcription factors. *J. Biol. Chem.* 272, 17795–17801.
27. Hauses, M., Tonjes, R. R., Grez, M. (1998) The transcription factor Sp1 regulates the myeloid-specific expression of the human hematopoietic cell kinase (HCK) gene through binding to two adjacent GC boxes within the HCK promoter-proximal region. *J. Biol. Chem.* 273, 31844–31852.
28. Eichbaum, Q., Heney, D., Raveh, D., Chung, M., Davidson, M., Epstein, J., Ezekowitz, R. A. (1997) Murine macrophage mannose receptor promoter is regulated by the transcription factors PU.1 and SP1. *Blood* 90, 4135–4143.
29. Chen, H. M., Pahl, H. L., Scheibe, R. J., Zhang, D. E., Tenen, D. G. (1993) The Sp1 transcription factor binds the CD11b promoter specifically in myeloid cells in vivo and is essential for myeloid-specific promoter activity. *J. Biol. Chem.* 268, 8230–8239.
30. Coleman, D. L., Bartiss, A. H., Sukhatme, V. P., Liu, J., Rupprecht, H. D. (1992) Lipopolysaccharide induces Egr-1 mRNA and protein in murine peritoneal macrophages. *J. Immunol.* 149, 3045–3051.
31. Tasanen, K., Oikarinen, J., Kivirikko, K. I., Pihlajaniemi, T. (1993) Interaction of transcription factor Sp1 with the promoter of the gene for the multifunctional protein disulphide isomerase polypeptide. *Biochem. J.* 292, 41–45.
32. Mummaneni, P., Yates, P., Simpson, J., Rose, J., Turker, M. S. (1998) The primary function of a redundant Sp1 binding site in the mouse aprt gene promoter is to block epigenetic gene inactivation. *Nucleic Acids Res.* 26, 5163–5169.
33. Biragyn, A., Nedospasov, S. (1995) Lipopolysaccharide-induced expression of TNF- α gene in the macrophage cell line ANA-1 is regulated at the level of transcription processivity. *J. Immunol.* 155, 674–683.
34. Yankulov, K., Blau, J., Purton, T., Roberts, S., Bentley, D. L. (1994) Transcriptional elongation by RNA polymerase II is stimulated by transactivators. *Cell* 77, 749–759.
35. Sweet, M. J., Hume, D. A. (1996) Endotoxin signal transduction in macrophages. *J. Leukoc. Biol.* 60, 8–26.