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A Ser/Thr Specific Protein Kinase Activates the Mouse Rantes Gene after Lipopolysaccharide Stimulation

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Macrophages stimulated by lipopolysaccharide (LPS) from gram negative bacteria undergo activation of a group of immediate early genes including Rantes. The mouse Rantes gene promoter region contains an LPS responsive element (LRE). We detected 3 specific bands, termed B1, B2, and B3, formed by the interaction of the LRE and proteins found in LPS-stimulated RAW 264.7 cells. An additional band, B4, was determined to be an AP-1 binding protein. The B1 band appears within 1 hour of LPS stimulation. The observed binding pattern could be changed by a specific heparin column fraction of nuclear extracts from LPS-stimulated cells. We have determined that a Ser/Thr-specific protein kinase is activated by LPS stimulation, and this protein kinase enhances B1 band formation. The B1 band can be converted to band B2/B3 by adding specific heparin column fraction. Purified Ser/Thr-specific protein phosphatases PP-1 and PP-2A can stimulate the same conversion to about the same extent. Thus, the formation of the LRE sequence binding complex appears to be regulated by Ser/Thr protein kinase and one or more Ser/Thr specific phosphatases. At least four proteins are involved in the regulation of the LRE-dependent Rantes expression: two binding factors that bind directly to the target sequences, and two factors that control their binding. The future purification and characterization of these binding proteins will reveal in detail the mechanism of Rantes gene activation after LPS stimulation.

Key words: mouse Rantes gene promoter, LPS responsive element, protein kinase and phosphatase

Infection of mammals by gram negative bacteria results in the specific activation of macrophages, including monocytes, by the bacterial cell wall component lipopolysaccharide (LPS) (26). Overstimulation with LPS can flood the area with potent proinflammatory cytokines such as IL-1, IL-6 and TNF-alpha (2, 26, 28, 30). This can cause endotoxic shock to various organs (9, 28). Macrophages carry out this potent antibacterial response in two ways. For the MHC class II deficient tissue macrophages, the CD 14 receptor binds LPS and this binding stimulates the cells (10, 41); this stimulation is enhanced as much as 1,000-fold by prior complexing of LPS with LPS binding protein (LBP) (3, 10, 21, 38). In the MHC class II expressing macrophages, LPS stimulated macrophages through the CD 14 secrete costimulatory molecules which induce primary T-cell responses. In addition, all macrophages can phagocytose target cells opsonized by complement or antibody (22), destroy target cells in a T-cell dependent manner, and induce antibody production. Thus, the recognition of LPS and subsequent responses of macrophages play critical roles in natural and specific immune reactions.

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In macrophages, the murine Rantes gene is activated by LPS stimulation within a few hours (36). As a member of the immediate early genes, Rantes may have significant effects on early stages of an immune reaction. Rantes is a member of the C-C subfamily (33, 40) of chemokines and functions as a proinflammatory chemoattractant for $CD4^+$ T cells (34, 37), monocytes, and eosinophils (19), and as an activator of histamine release in basophils (23). The murine Rantes gene is located on chromosome 11. It is a single copy gene composed of 3 exons spanning approximately 4.5 kb (7, 13, 35). The amino acid sequence of murine Rantes is 90% identical to its human counterpart.

To understand the molecular mechanisms of LPS-induced gene activation, Shin et al. compared the promoters of the mouse Rantes gene and the crg-2 gene which is another immediate early LPS-activated gene (36). Comparison of these two promoters revealed that the LPS responsive element (LRE) of the mouse Rantes gene consists of two conserved motifs. One motif is an AP-1 half site with two flanking bases, and the other resembles a portion of the interferon-stimulated responsive element (ISRE) (36). These two motifs are separated by 10 non-consensus nucleotides.

Electrophoretic mobility shift assays (EMSAs) of the

Rantes LRE detect 3 specific bands, designated as B1 (slow migrating), B2 and B3 (detected as a middle migrating doublet). The B1 band is greatly enhanced after LPS stimulation and reaches its highest level within 1 h. We know that the observed binding patterns can be changed by column fractions of nuclei of LPS-stimulated cells. Thus it appears likely that these column fractions contain a protein kinase which is associated with Rantes activation.

In this paper, we have characterized proteins binding to the mouse Rantes promoter region (-192 to -118) using nuclear extracts derived from LPS stimulated or unstimulated RAW 264.7 macrophage-like cells. Fraction reconstitution assays show that at least two factors bind to the target sequences directly, and their binding or activity is controlled by a protein kinase (PK) and a Ser/Thr specific protein phosphatase. Our data support a model in which LPS activates a Ser/Thr PK, which phosphorylates the putative binding proteins that bind with high affinity to the LRE and activate transcription of the murine Rantes gene. The process is reversed by a protein phosphatase whose activity resembles type-1 (PP-1) and type 2A (PP-2A) protein phosphatases.

Materials and Methods

Cell cultures and nuclear extract preparation

The mouse macrophage-like cell line RAW 264.7 was obtained from ATCC (Rockville, MD, USA), and maintained in RPMI 1640 medium containing 10% fetal bovine serum, 100 μ/ml penicillin, 100 mg/ml streptomycin, and 2 mM glutamine at 37°C under 6% CO_2. For the stimulation experiments, cells were plated in growth medium at 70% confluence overnight. After a 2 h incubation in serum-free medium, the cells were incubated with *E.coli* LPS (1 μg/ml, Sigma) for 1 to 4 h.

Nuclear extracts were prepared by the modified procedure of Dignam *et al.* (8). Cells were stimulated with LPS for 1 or 2 h at 37°C under 6% CO_2, then scraped and washed with ice cold PBS (137 mM NaCl, 2.7 mM KCl, 10.6 mM Na_2HPO_4, and 1.4 mM NaH_2PO_4). The packed cells were washed once with 50 volumes of hypotonic buffer (20 mM Hepes pH 7.5, 5 mM KCl, 1.5 mM $MgCl_2$, and 1 mM DTT), then resuspended in 10 × volumes of the same buffer. After swelling on ice for 30 min, the cells were disrupted with a Dounce homogenizer (30 strokes, B pestle) until more than 90% of the cells were lysed. Lysates were separated by centrifugation at 13,000 rpm for 15 min, then the pellets were resuspended with elution buffer SWBB [0.4M NaCl, 20 mM Hepes pH 7.5, 4 mM $MgCl_2$, 1 mM EDTA, 0.1 mM $ZnSO_4$, 1 mM DTT, 5% glycerol, and protease inhibitor cocktail solution (Boehringer Mannheim)]. Lysates were stirred for 30 min at 4°C with a mini magnetic bar. Clustered nuclei were removed by centrifugation for 15 min at 12,000 × g. The supernatant was dialyzed for 2 h at 4°C against dialysis buffer (20 mM Hepes pH 7.9, 20% glycerol, 0.1M KCl, 0.2 mM EDTA 0.5 mM PMSF, and 1 mM DTT) and stored in aliquots at -80°C. We obtained 0.5-1 mg of protein by this method.

Probe preparation and EMSA

Two 70-base complementary oligonucleotides, containing the sequence spanning from -192 to -118 of the Rantes promoter, were chemically synthesized and annealed. The annealed double stranded oligonucleotide had a 3-base overhang at the 5'-end of the positive strand (GGG) and a 4-base overhang the 5' in the negative strand (TTTT). The overhanging strands were labeled by filling in with a [α-^{32}P] dCTP and [α-^{32}P] dATP using the Klenow fragment of DNA polymerase I. The probes were electrophoresed on a 3% low melting point agarose gel. The agarose gel fragment containing the probe was melted at 65°C, then extracted once with phenol, once with chloroform, and once with phenol/chloroform/isoamylalcohol (25:24:1). The probe was reprecipitated with ethanol and dried using a speed vacuum drier. The purified probe was reannealed and the specific activity (10^8cpm/μg) was estimated by scintillation counting. The oligonucleotide sequence used to make the wild-type probe was; 5'-GGG CAG TTA GAG G*AG AGT CA*T ACT TCC AAG GGT *GAT TTC AGT TTT CTT TTC CAT TTT GTG TTT TCA TTT T*-3'; and this sequence includes the AP-1 (6 fit among 7 bases) and T-rich region.

Five μg of nuclear extract was incubated for 5 min at 21°C with 3 μg poly (dI-dC) (Pharmacia) or poly (dG-dC) (Pharmacia) in SW binding buffer (20 mM Hepes pH7.5, 50 mM NaCl, 4 mM $MgCl_2$, 1 mM EDTA, 0.1 mM $ZnSO_4$, 1 mM DTT, and 5% glycerol). The reaction mixtures were incubated for 15 min at 15°C with labeled probe (30,000 cpm) in a final volume of 20 μl. The mixtures were electrophoresed on 3% stacking and 7.5% running discontinuous or 7.5% continuous polyacrylamide gels under 0.4X TBE buffer (35.6 mM Tris-borate pH 8.0, 0.8 mM EDTA).

Column fractionations

Frozen stocks of RAW 264.7 nuclear extract were thawed and centrifuged at 12,000 ×g for 10 min. The supernatant was loaded onto a pre-packed heparin column (1 ml, Pharmacia) equilibrated with 50 mM NaCl SW binding buffer. For the stepwise elution, the column was washed with 3 column volumes of the equilibration buffer, and proteins were eluted with 3 column volumes of each of 0.2, 0.4, and 0.6 M NaCl in SW binding buffer. For the glycerol gradient, the nuclear extract of RAW 264.7 cells was fractionated by using a continuous gradient (5 ml, 15-35%) in buffer T (25 mM Tris-Cl pH 7.8, 0.15 M NaCl, 1 mM EDTA, 1 mM DTT). After centrifuging at 200,000

×g for 24 h, fractions (100 μl) were collected from the top of the gradient tube and assayed for binding activity using the EMSA system. LRE binding factor and converting/enhancing factor fractions were plotted against standard protein markers: bovine serum albumin, aldolase, and catalase. From the standard plot, the sedimentations and approximate molecular weights were calculated. All purification steps were performed at 4°C. For reconstitution experiments, each of the heparin column fractions was incubated for 5 min at room temperature before adding the probe, then incubated for 15 min at 15°C with the probe.

Protein phosphatase and kinase assays

To characterize the converting factor which converts the slow migrating band (B1) into the middle migrating bands (B2 and B3), the converting activity and band B1 containing fractions were incubated with various phosphatases for 15 min at 15°C prior to probe reaction in the presence or absence of okadaic acid. We examined calf intestinal alkaline phosphatase (CIP), protein phosphatase type 1 (PP-1), protein phosphatase type 2 (PP-2A), calcineurin (PP-2B), and protein tyrosine phosphatase 1B (PTP-1B). Reactions were examined by PAGE as described above (see EMSA). All phosphatase were purchased from Upstate Biotechnology, Lake Placid, NY, USA.

Protein kinase activity was investigated in those glycerol gradient fractions which enhanced B1 band formation and slowed its migration. Fractions were reacted with 2 μg myelin basic protein fragment (residue 4-14, Sigma) or histone (type III-SS: calf thymus, Lys-rich fragment, Sigma) in a reaction buffer containing 50 mM Tris-Cl pH 8.0, 10 mM Mg^{2+}, 1 mM ATP, and 5 μCi [γ-^{32}P] ATP for 20 min at 21°C. After incubation, 50 mM EDTA was added and the samples were heated and examined by 12% acid urea SDS-PAGE for MBP or 15% SDS-PAGE for histone, both under reducing conditions.

For the protein kinase inhibition assay, RAW 264.7 cell nuclear extracts were stimulated with LPS in the presence or absence of protein kinase inhibitor H7, HA1004, or H8 (Seikagaku, Japan). Protein kinases were treated with 60 μM of the inhibitor for 1 h prior to LPS stimulation. RAW 264.7 cells were harvested as described above, and the extract-probe reactions were electrophoresed by discontinuous PAGE.

Results

Optimization of EMSA

SW binding buffer, which contains slightly higher reagent concentrations than most EMSA binding buffers, gave the best results (see Materials and Methods). We also tested the nonspecific competitors poly (dG-dC), poly (dI-dC), and heparin. Three bands were formed in the presence of poly (dG-dC) (Fig. 1A). We also tested two gel electrophoresis systems: continuous PAGE and discontinuous PAGE. In situations where we needed fine resolution of the B2/B3 doublet, continuous PAGE gave better results. We designated the observed bands according to their migration rate; the slow band is B1, the middle doublet is B2/B3 (Fig. 1).

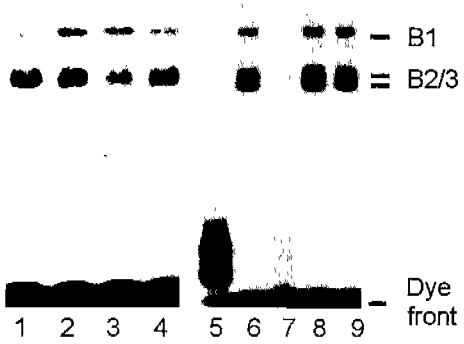

Fig. 1. Binding of nuclear factors derived from RAW 264.7 cells. Nuclear proteins from LPS stimulated RAW 264.7 cells were incubated with radio-labeled mouse Rantes oligonucleotides (0.1 ng) spanning nt -192 to -118. After incubation, the mixtures were analysed by 7.5% continuous PAGE. For time kinetics of B1 migration (lanes 1 to 4), nuclear protein stimulated with LPS reacted with specific probe. Lane 1, unstimulated nuclear extract; lane 2, 1 hour stimulation; lane 3, 3 hours stimulation; lane 4, 5 hours stimulation. For competition experiments (lanes 5 to 9), the speaific or nonspecific cold probes were added before adding specific radiolabeled probe. Lane 5, without nuclear extract; lane 6, LPS stimulated nuclear extract; lane 7, competition with 0.4 ng specific cold probe; lane 8, competition with 0.4 ng non-specific probe; lane 9, competition with 1ng non-specific probe. B1 and B2/B3 represent the slow and middle migrating bands, respectively. The highly migrated band in lane 5 is a free probe that was not bound to protein. Dye front is a free [α-^{32}P] dCTP or [α-^{32}P] dATP that was not bound to a probe.

The induction kinetics of LRE binding proteins

To determine the time kinetics of LPS stimulation on binding factor induction, serum starved RAW 264.7 cells were stimulated with LPS for 1, 3, or 5 h. The slow migrating band (B1) was induced within 1 h by LPS stimulation, and its presence continued for 6 h without any change in the binding pattern (Fig. 1, lanes 1 to 4).

We performed a competition assay in which nuclear extracts from LPS-stimulated RAW 264.7 cells were allowed to bind to the radiolabeled 70-bp LRE probe in the absence or presence of unlabeled competitors. The 70-bp probe alone revealed the three usual bands (Fig. 1, lane 6). Addition of 0.4 ng (4-fold excess) of cold specific probe nearly eliminated this binding (Fig. 1, lane 7). The same or 10-fold greater amount (1 ng) of linearized PUC118 plasmid DNA did not affect the binding (Fig. 1, lanes 8 and 9). These data show that LPS stimulation induces some binding factor (s), which greatly enhances the formation of the B1 band on EMSA.

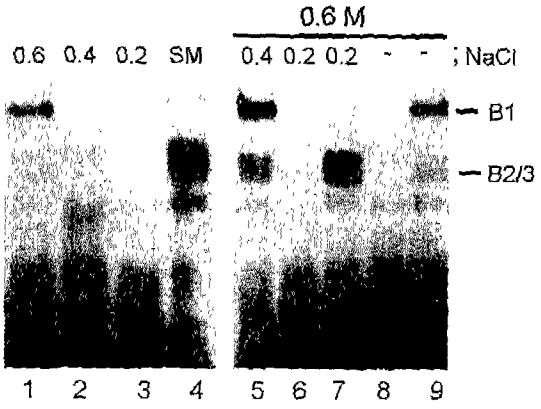

Fig. 2. EMSA pattern of fractions from step heparin column. LRE binding activities of the fractions were eluted from the heparin column with a stepwise NaCl gradient from 0.2 to 0.6 M in binding buffer. The mixtures were analysed by 7.5% continuous polyacrylamide gel in the 0.4X TBE buffer. Lane; 1, 0.6 M eluted fractions; 2, 0.4 M eluted fractions; 3, 0.2 M eluted fractions; 4, starting material (SM; crude nuclear extract before chromatography); 5, reconstitution of 0.6 and 0.4 M NaCl; 6, competition assay of 0.6 and 0.2 M reconstitution with cold probe; 7, reconstitution of 0.6 and 0.2 M NaCl; 8, competition of 0.6 M fraction with specific cold probe; 9, 0.6 M NaCl eluted fraction only.

Heparin columns and fraction reconstitution

Samples of 5 mg extract were loaded on a 1 ml heparin column then eluted stepwise in 0.2, 0.4, 0.6, and 1.0 M NaCl in SW binding buffer (Fig. 2). Band B1 and faint B2/3 bands were specifically formed by factor(s) in the 0.6 M NaCl fraction (Fig. 2, lane 1). This experiment suggests that the B1 and B2/3 bands are correlated with each other because these three bands are associated with the same eluted fractions. Further evidence of the correlation of the B1, B2, and B3 bands was also provided by reconstitution assays.

During preliminary experiments, we noticed that certain column fractions, when mixed with the 0.6 M-eluted bands, could convert B1 into B2/B3 or enhance B1. The 0.2 M NaCl fraction contains a conversion factor which converts band B1 to B2/B3 bands (Fig. 2, lanes 5 to 8). The converted B2/3 bands were eliminated by adding a 5-fold specific cold probe (Fig. 2 lane 6). Additional experiments were performed to determine whether these converting effects are general phenomena. Using the linear gradient fraction which contained the B1 and B2/B3 band formation factor (s), addition of the 0.2 M fraction from the stepwise elution could, again, convert B1 to B2/B3 bands (data not shown). The enhancing factor activity could also be detected in another reconstitution experiment. Heparin column fraction (Fig. 2, lane 1) eluted at 0.6 M NaCl was mixed with glycerol gradient fractions. Reconstituted with glycerol gradient fractions 13 and 17 gave the strongest enhancing activity (Fig. 3, lanes 5 and 6). To rule out the possibility that salt concentration was causing the observed changes in the band patterns, the 0.6 M fraction was assayed with buffers of varied salt concentrations. Adding equivalent amounts of 0.6 M or 0.2 M NaCl containing buffers did not change the EMSA patterns of the 0.6 M fractions (data not shown). Thus we conclude that there is a converting factor and an enhancing factor that can affect the binding of the band formation factor (s) to the Rantes LRE (Fig. 2 and 3).

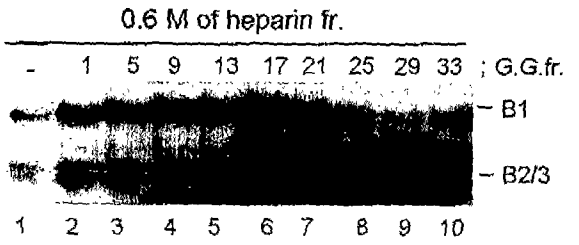

Fig. 3. Reconstitution assay with heparin column fractions. The 0.6M eluted fraction from the heparin column was reconstituted with glycerol gradient fractions, and the reactions were analysed by EMSA. Letters above the panel indicate the number of each glycerol gradient fraction.

Determination of the sedimentation coefficient of the converting and enhancing factors

To further characterize the LRE-binding protein(s), we performed fractionation by glycerol gradient. All fractions were tested by EMSA. The B1 and B2/B3 bands were found in fractions 35-37 and 23-25 (our previous work). The sedimentation coefficient and approximate native molecular weights of the middle and slow bands were determined to be 8.0S (about 140 kDa) and 12.0S (about 270 kDa), respectively. We were also able to estimate the sedimentation coefficient and native molecular weight of the converting and enhancing factors with a reconstitution assay using glycerol gradient fractions. To determine the sedimentation coefficient of the converting factor, heparin 0.6 M-eluted fraction (Fig. 2, lane 1) was mixed with each of the glycerol gradient fractions (Fig. 3). The peak of converting factor activity was around fraction number 30 (Fig. 3, between lanes 9 and 10). The sedimentation coefficient for this glycerol gradient fraction corresponded to 6.3S (120 kDa). Likewise, the enhancing factor that enhances and retards band B1 (Fig. 3, between lanes 5 and 6), was estimated at fraction number 16 (4.4S or 70 kDa).

Characterization of the converting factor(s)

The B1 complex (Fig. 4A, lanes 1 and 2) was completely converted to B2/B3 after addition of one-half volume of the converting factor containing fraction (Fig. 2, lane 3). We speculated that this migration difference resulted from phosphate modification of the binding factor. After assaying a number of phosphatases, we found that protein phosphatase type 1 (PP-1) and protein phosphatase type 2A (PP-2A) could convert the B1 band to the B2/B3 band (Fig. 4B). Both PP-1 and PP-2A are specific for phosphoserine and phospho-threonine but not for phosphotyrosine. PP-1 showed stronger converting activity than

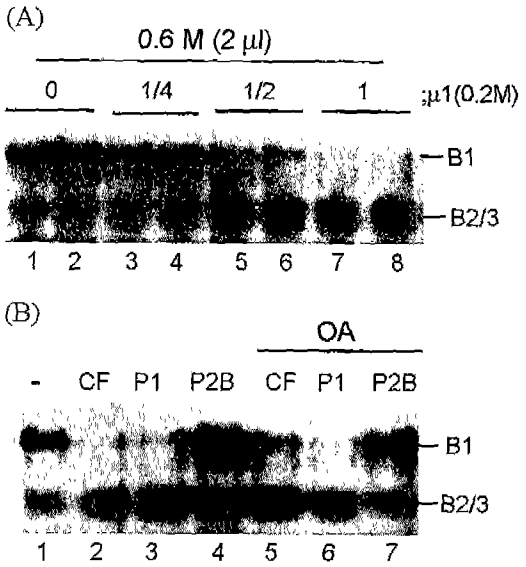

Fig. 4. Characterization of converting factor. (A) The 0.6 M eluted fraction from the heparin column was reconstituted with increasing amounts of 0.2 M fractions, and the reactions were analysed by EMSA. Letters above the panel indicate the volume of 0.2 M NaCl fraction. (B) Heparin column 0.6 M fraction was incubated with C.F. (lane 2 and 5), protein phosphatase type-1 (lane 3 and 6) and protein phosphatase 2B (lane 4 and 7) in the presence (lanes 5-7) or absence (lanes1-4) of 40 nM okadaic acid for 15 min at 15°C. After the probe reaction, they were incubated at 15°C for another 15 min. Lane 1 is the control without phosphatase.

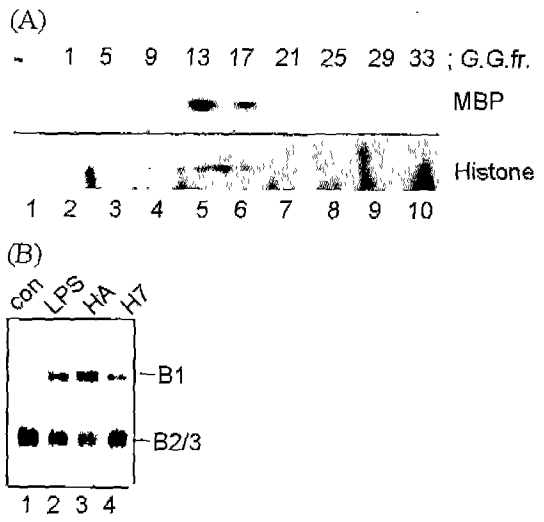

Fig. 5. Protein kinase and inhibition assay. (A) Glycerol gradient fractions were incubated with histone or myelin basic protein. After 50 mM EDTA was added to reaction mixtures, they were separated on 15% SDS-PAGE for histone and 12% acidic urea SDS-PAGE for MBP under reducing conditions. (B) RAW 264.7 cells were stimulated with LPS in the presence or absence of protein kinase inhibitors H7 or HA1004. The extract-probe reactions were separated by discontinuous PAGE. Lanes: 1, without LPS stimulation; 2, LPS stimulation (1 μg/ml); 3, HA1004 treatment prior to LPS stimulation; 4, H-7 treatment prior to LPS stimulation.

PP-2A when equal units of the two enzymes were used (data not shown). But calcineurin (P-2B; Fig. 4B, lane 4), which has Ca^{2+} or Mn^{2+} dependent phosphoprotein phosphatase activity toward phospho-tyrosine, did not affect the B1 band. 40 nM okadaic acid partially inhibited the converting activity of the crude converting factor (Fig. 4B, lane 5), but did not inhibit the activity of affinity purified PP-1 (Fig. 4B, lane 6).

Characterization of the enhancing factor

Enhancing activity was detected in reconstitution experiments using heparin and glycerol gradient fractions (Fig. 3, lanes 5-6). The fractions were tested for protein kinase activity as shown Fig. 5A. Myelin basic protein (residues 4-14) is a protein kinase C substrate. Histone is a suitable substrate for protein kinase C and other protein kinases. Fractions 13-17 from the glycerol gradient demonstrated both potent protein kinase C activity (Fig. 5A, lanes 5 and 6) as well as enhancing activity for B1 (Fig. 3, lanes 5 and 6).

To assay protein kinase inhibition, RAW 264.7 cells were treated with H-7, HA1004, or H-8 prior to LPS stimulation (Fig. 5B). H-7 is the most potent and selective protein kinase inhibitor, and it is an essential tool for the elucidation of the role of protein phosphorylation by protein kinase C (17, 20). HA 1004 is the weakest protein kinase C inhibitor and is useful as a control for H-7 (4, 14). H-8 has markedly higher affinities for cycle nucleotide-dependent protein kinases (14). H-7 decreased B1 band formation about 40% (Fig. 5B, lane 4). The quantitation of band formation was measured by densitometry.

Discussion

Macrophages can be activated by a variety of agents. As a step toward understanding the molecular mechanisms of LPS induced immediate early gene activation, we investigated the nature of the LRE of the mouse Rantes gene; Rantes is activated by LPS in the absence of protein synthesis. Shin *et al.* used deletion analysis to identify the essential components that make up the minimal LRE, distinct from the regulatory sequences that can enhance or repress LRE activity (36). Within the 5' untranslated region, they identified three conserved sequences designated as motifs 1, 2, and 3. Also, they demonstrated by mutational analysis that motifs 1 and 3 are needed for LRE activity. In this study, we focused our effort on characterizing the essential binding and regulatory factors using an EMSA system. Using a 70bp probe (-192 to -118), we identified a pattern of three bands (B1 to B3) which reveal the presence of at least three binding factors. Among the three complex-forming factors, the slowest migrating band, B1, increased dramatically following LPS stimulation (as shown Fig. 1).

To further characterize the binding mechanism, we frac-

tionated the extract and performed reconstitution assays. We identified the converting factor that converts B1 to B2/B3 bands. This converting activity resembles the activity of nuclear protein Ref-1, which maintains the reduced state of the cysteine residue located in the DNA binding domain of Fos and Jun, and thus promotes their binding to AP-1 sites (42-44). The LRE target sequence contains an AP-1 (6/7) site in its 5' region. But our immunoblotting and immunodepletion data showed that anti Ref-1 did not affect the formation of B1 band (data not shown). We determined that the converting factor is likely to be a kind of protein phosphatase type 1 or 2. These phosphatases are highly specific for serine and threonine-linked phosphates (15).

We have also shown that the enhancing factor is a protein kinase C. It is able to phosphorylate the Ser of myelin basic protein, which is the most specific and convenient substrate for PKC (45). A significant increase in PKC activity has also been observed in macrophages following treatment with gamma interferon, endotoxin-associated protein, or *E. coli* lipid A precursor (1, 11, 39).

Mammals have as many as 2,000 PK genes (36) and 1,000 PP genes (15). They control a wide range of cellular events, such as cell division, cell signaling, differentiation, and metabolism (29, 30). Moreover, in many cases, individual enzymes are involved in the regulation of several different processes. Recent studies have also shown that protein phosphatase and kinase are translocated to the nucleus dynamically during cellular events. A translocation process of certain molecules is reversibly controlled by protein kinase and protein phosphatase (15, 24). The importance of PPs in cellular physiology is emphasized by the fact that they are often targets for microbial toxins (27). Many microbial toxins are inhibitors of PP-2A, or the closely-related PP-1, or both (5, 12, 18, 25). And okadaic acid is a strong inhibitor of PP-1 and a potent inhibitor of PP-2A (6). These data support our findings that LPS activates a PKC, and the PKC phosphorylates binding factors which activate Rantes.

Acknowledgments

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