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Restoration of Lipopolysaccharide-Mediated B-Cell Response after Expression of a cDNA Encoding a GTP-Binding Protein

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Previous analysis of hybrid progeny derived from lipopolysaccharide (LPS) responder and nonresponder inbred mouse strains demonstrated that a single genetic locus controlled responsiveness to LPS. Using a differential functional screening approach, we report the isolation of a cDNA that has sequence homology to a GTP-binding protein. Expression of the cDNA in splenic B cells of C3H/HeJ nonresponder, endotoxin-resistant mice resulted in polyclonal B-cell activation in response to LPS stimulation. Thus a GTP-binding protein may be involved in LPS stimulation in B cells and perhaps other cell types.

The underlying genetic basis for the multiple responses of the host to lipopolysaccharide (LPS) endotoxins was initially defined with the discovery of the C3H/HeJ mutant mouse strain in 1968 (23). The strain is hyporesponsive to the immunostimulatory and pathophysiological effects of the lipid A component of LPS and, therefore, is considered to have a fundamental deficiency in its reactions to LPS compared with closely related responder strains.

This defect is specific and expressed in a variety of ways. For example, C3H/HeJ B cells do not proliferate or differentiate when exposed to LPS, and the proliferation of their thymocytes induced by concanavalin A is not enhanced by LPS (11, 17, 24–29). Macrophage phagocytosis and cytotoxicity are not stimulated by LPS, and the cytokines such as tumor necrosis factor, interferon, colony stimulating factor, and interleukin-1 (IL-1) and the prostaglandins stimulated by LPS in responder cells are not induced in C3H/HeJ macrophages (17). Furthermore, the C3H/HeJ mouse is highly resistant to lethal endotoxin shock compared with normal endotoxin responder strains (23).

The failure of C3H/HeJ cells to respond to LPS is not due to the absence of helper cells or the presence of suppressor cells or for that matter the deficient binding of LPS to otherwise immunocompetent cells (26). Rather, the explanations offered focus on a deficient trigger receptor or a failure somewhere in signal transduction after the initial interaction of the cells with LPS (28).

From the results of classical-type breeding experiments with the C3H/HeJ strain and various responder strains, it has been found that the mitogenic response to LPS is governed by a single locus composed of codominant alleles (11, 17, 25, 26, 28). Watson et al. determined that the locus (*Lps*^{n}) was on chromosome 4 and linked to the major urinary protein locus (*Mup-1*) but downstream from *Mup-1* and the *Lyb2;2;4;6* genes which control B-cell activation (34). As a consequence of these findings, it is generally believed that if the product and function of the *Lps*^{n} gene can be found, the key to a clearer understanding of how LPS works at the molecular, cellular, and host levels will be at hand. In this study, using a novel functional screening method, we have isolated a cDNA whose expression in C3H/HeJ splenic B cells resulted in their responsiveness to LPS stimulation.

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MATERIALS AND METHODS

Mice and cells. LPS responder inbred mice C3H/HeOuJ and nonresponder mice C3H/HeJ were purchased from the Jackson Laboratory. Primary spleen cells were collected as described before (23, 25).

DNA and RNA extraction and cDNA library construction. Extraction of genomic DNA and total RNA was done according to the method described by Sambrook et al. (22). The construction of the cDNA library was as described previously (35) and was modified from the method of Okayama and Berg (19). The source of mRNA for the cDNA library derived from LPS-stimulated spleen cells was obtained from C3H/HeOuJ mice. Conditions for LPS stimulation were as described by Sultzer (23, 25).

Plaque assay. The C3H/HeJ spleens were removed and placed in cytotoxic medium (CyM; Cedarlane Labs), and erythrocytes were lysed by treating the cells with 0.75% NH_4Cl twice. T cells were removed by treatment with anti-Thy1.2 antibody (Sigma) at a 1:10,000 dilution in the presence of a Low Tox-M rabbit complement (Accurate Chemical Co.) at 37°C. The cells were then washed with R5 (RPMI 1640 with 5% fetal bovine serum) and resuspended at 3×10^7 viable cells per ml. One milligram of C3H/HeOuJ-derived sublibrary plasmid cDNA was added to 1 ml of resuspended cells and transferred to an ice-cold 0.4-cm-diameter Gene Pulser cuvette. The mixture was electroporated at 250 V and 960 μF with a BioRad Gene Pulser and incubated on ice for 10 to 30 min. These electroporated cells were then stimulated with *Salmonella typhi* LPS (100 μg/ml) and incubated for 72 h in an incubator containing 7% O_2–10% CO_2 and balanced with N_2. Each day, the cells were fed with R5. After 72 h of incubation, the cells were harvested and washed in MSS buffer solution. A total of 200,000 viable cells were mixed with 10% sheep erythrocytes suspended in Alservers buffer (Organon Teknika, Cappel) and liquified 0.7% agarose in MSS buffer (pH 7.0–7.2) (NaCl, 8 g; KCl, 0.4 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; $MgCl_2 \cdot 6H_2O$, 0.2 g; KH_2PO_4, 0.06 g; $Na_2HPO_4 \cdot 7H_2O$, 0.36 g; and $CaCl_2$, 0.14 g [all per liter]) and plated on a solidified 0.7% agarose plate. These plates were then overlayered with guinea pig complement at a 1:15 dilution and incubated in 7% O_2–10% CO_2 and balanced with N_2 for 2 to 3 h, after which time the numbers of plaques were recorded.

RESULTS

We have engaged in studies to clone this putative gene (28). We anticipated that a functional gene product must be present in polyclonally activated antibody-producing B cells from LPS responder mice such as the C3H/HeOuJ. Introduction of this cDNA (*Lps*^{n}) into C3H/HeJ B cells should restore their responsiveness to LPS stimulation, resulting in the increase of

TABLE 1. LPS-stimulated PFCs after electroporation of pCD library DNA[a]

DNA	Electro-poration	Stimulant	PFC/10 dishes	Plasmid DNA/negative control ratio
LB2.3	+	LPS	7	0.78
None	+	LPS	9	
None	−	EP	TMTC	
LB2.4	+	LPS	11	1.38
None	+	LPS	8	
None	−	EP	TMTC	
LB2.5	+	LPS	7	0.58
None	+	LPS	12	
None	−	EP	TMTC	
LB2.2	+	LPS	22	5.5
None	+	LPS	4	
None	−	EP	TMTC	

[a] Since a large number of cells were needed for each point, electroporation of spleen cells with each sublibrary plasmid DNA was done separately at different times. Because of that, negative control (no DNA but still stimulated with LPS) and positive control (no DNA but stimulated with EP) were incorporated each time. +, electroporation performed; −, no electroporation performed; EP, endotoxin-associated protein which activates C3H/HeJ cells (6); TMTC, too many to count.

antibody-producing plaque-forming cells (PFCs) to a responder level. To show that, we first extracted mRNA from LPS-stimulated C3H/HeOuJ spleen cells after the removal of erythrocytes and T cells (see Table 1). From these mRNA, a cDNA pCD plasmid library was constructed by using the Okayama-Berg expression vector (19). The library consisted of six sublibraries, with sizes ranging from 1×10^4 to 2×10^4 independent clones.

To introduce the pCD library DNA into cells, we used electroporation. Because the efficiency of gene transfer by this technique is highly variable, we performed some standardization experiments with immortalized pre-B 70Z/3 lymphoid cells. Under standardized optimal electroporation conditions, we showed that the efficiency of gene transfer is about 1 in 10,000 cells electroporated. Following the experimental approach described in the legend to Fig. 1, we then electroporated sublibraries of plasmid DNA into C3H/HeJ spleen cells enriched with plaque-forming B cells. The results in Table 1 suggest that electroporation of spleen cells with plasmid DNA from sublibrary 2.2 gave a higher number of plaques compared with those with other sublibrary DNA or with no plasmid DNA at all. We therefore subdivided sublibrary 2.2 into 4 sublibraries and repeated the analysis. This time, sublibrary 2.2.D was positive over other plasmid DNA and negative with no DNA at all.

By progressively reducing the number of independent clones in each sub-sublibrary from a particular positive sublibrary and by using this assay to isolate cDNA by its function, we were able to detect after each level of subdivision one sublibrary which consistently tested positive. In addition, the ratio of PFCs from the "positives" over those of the "negatives" increased as the number of independent colonies in each sublibrary decreased (Fig. 1). These data are consistent with the idea that the enrichment of one particular clone of plasmid DNA accounts for the increase in the numbers of PFC detected. Indeed, we showed that LB2.2.D.1.B.3.S plasmid preparation contained a single species of plasmid DNA; its introduction and expression in C3H/HeJ spleen cells led to an increase in the numbers of PFCs. We have therefore shown that the deficient C3H/HeJ cells can be stimulated by LPS after expression of this cDNA. We call this plasmid pCD-LPS and the cDNA, *LPS* (Fig. 2).

To further confirm that the expression of the putative LPS-responding cDNA resulted in LPS-stimulated plaque formation by electroporated C3H/HeJ spleen cells, we reanalyzed plasmid preparations from various cDNA sublibraries. These plasmids were digested with *Xho*I, an enzyme whose recognition sites flank all cDNA inserts at both ends in the pCD vector. The *Xho*I recognition sequence is rarely represented in the mammalian genome, and therefore frequency of the *Xho*I site within the cDNA is low. After *Xho*I digestion, the DNA was fractionated in a 1% agarose gel and transferred and fixed onto a nylon filter, which was then hybridized with an *Lps*^{n}-specific probe. After hybridization and washing and exposing the filter to an X-ray film, we observed that all of the sublibrary plasmid DNA that tested positive by the plaque assay produced a 1.5-kb signal, whereas those tested negative by the plaque assay did not (Fig. 3). In addition, the intensity of the signal was proportional to the estimated representation of pCD-LPS (Fig. 3). These data show that the expression of

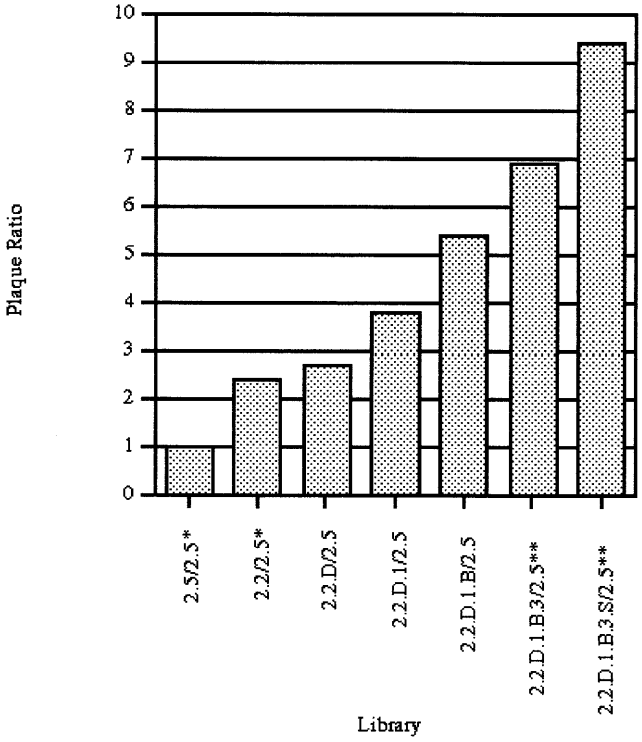

FIG. 1. Functional screening by using a modified PFC assay. We performed the modified Jerne's plaque-forming assay as described in Table 1. Initially, six sublibraries (LB2.1 to LB2.6) were constructed from LPS-stimulated spleen cells from C3H/HeOuJ mice. The size of each sublibrary ranged from 1×10^4 to 2×10^4 independent clones. After testing positive on the PFC assay LB2.2 (containing 2×10^4 independent clones), but not other sublibraries, was subdivided further into four sub-sublibraries (LB2.2.A to D), each of which had 6,000 independent clones. Among those, LB2.2.D tested positive and was therefore divided further into 10 sublibraries, LB2.2.D.1 to LB2.2.D.10, each of which had 600 independent clones. LB2.2.D.1 tested positive and was subdivided into six sub-sublibraries, LB2.2.D.1.A to -F, with 100 pooled clones each. Among them, LB2.2.D.1.B was positive and was then divided into 10 pools of sublibraries with 10 independent clones per sublibrary. Of these sublibraries, LB2.2.D.1.B.3 tested positive. After further division into 10 single clones, LB2.2.D.1.B.3.S tested positive and was subsequently named pCD-*Lps*^{n}. *, To explain the data more consistently, the data were normalized on the basis of the background values from the plaque ratios of 2.5/no DNA. These two experiments were done with the same batch of mice. **, For consistency, the data from one experiment that included the 2.2.D.1.B.3 sublibrary were normalized to the data from another experiment that included the 2.2.D.1.B, 2.2.D.1.B.3, and 2.2.D.1.B.3.S sublibraries. Therefore, these two ratios are normalized on the basis of the 2.2.D.1.B sublibrary.

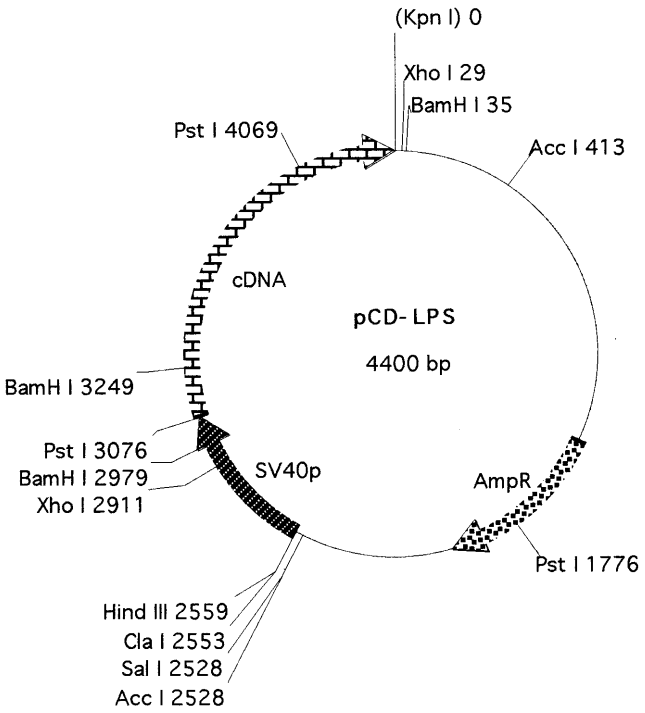

FIG. 2. Restriction map of pCD-LPS. The size of the cDNA is about 1.35 kb. SV40p is the simian virus 40 promoter. Amp^r represents the ampicillin resistance gene. The numbers after the abbreviated restriction enzymes are map positions of the recognition sites.

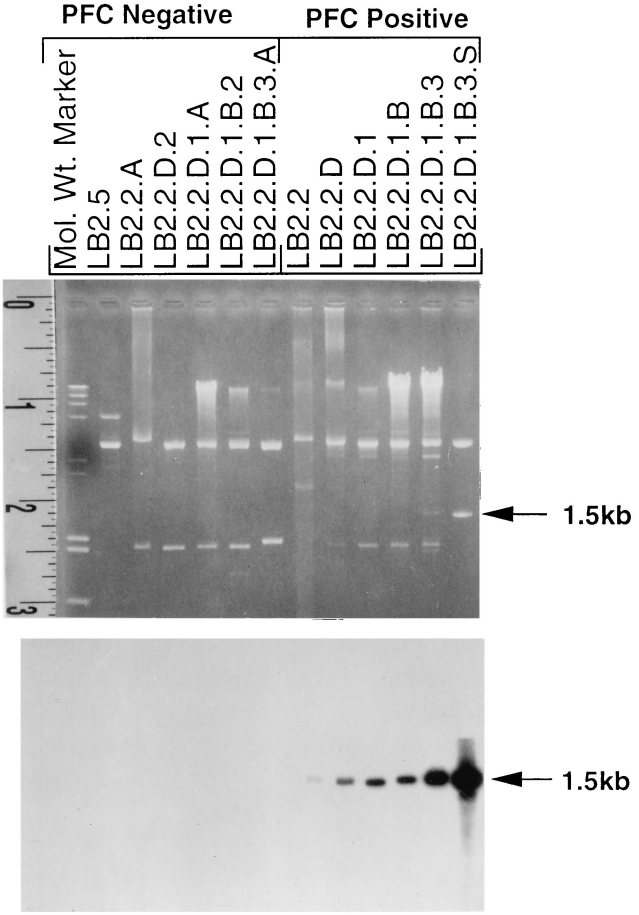

FIG. 3. Hybridization of positive and negative sublibraries with pCD-LPS cDNA. Plasmid DNA was extracted from bacteria of various pCD sublibraries made from LPS-stimulated C3H/HeOuJ spleen cells (28). DNA (5 to 10 μg) was digested with *Xho*I, which has two unique sites flanking all cDNA inserts in the pCD vector. After digestion, the DNA was fractionated by agarose gel and stained with ethidium bromide (upper panel). The DNA in the gel was then transferred to a nylon filter, fixed by UV cross-linking, hybridized with a [^{32}P]dCTP-labeled probe from a 0.8-kb *Bam*HI-*Pst*I DNA fragment specific to the *LPS* cDNA, and exposed to an X-ray film (lower panel). PFC Negative or PFC Positive is the group of plasmid DNA from sublibraries that tested negative or positive by the plaque assay. LB2.2 was the PFC-positive sublibrary, LB2.2.D was the PFC-positive sub-sublibrary, etc. Note the presence of a 1.5-kb band in all PFC-positive sublibraries but not in all PFC-negative sublibraries. The intensity of the band increased progressively as the results of the PFC assay became more positive (see Fig. 1).

pCD-LPS cDNA in C3H/HeJ mice accounts for the LPS-stimulated plaque formation by polyclonal-activated B cells.

Next we performed additional experiments confirming that this *LPS* gene is involved in restoring LPS responsiveness. The pCD-LPS plasmid DNA was introduced into splenic B cells of C3H/HeOuJ responder mice and of C3H/HeJ nonresponder mice by electroporation as before, and the cells were used for plaque assays. The results are tabulated in Table 2. They show that C3H/HeJ cells electroporated with pCD-LPS produced a baseline number of plaques in the absence of LPS (8 plaques) but produced a significantly higher number in the presence of LPS (37 plaques). Cells electroporated with a non-LPS plasmid DNA, LB2.5, produced plaques in numbers not different from the background (6 plaques). Electroporation apparently reduced the number of PFC preferentially (from 151 to 50 plaques for C3H/HeOuJ cells, in the presence of LPS). Even in the face of this reduction effect of electroporation in the LPS responder cells, the C3H/HeJ nonresponders were activated after electroporation of the LPS cDNA. Cells from C3H/HeOuJ mice responded to LPS, whether they were electroporated with pCD-LPS or with LB2.5, albeit the stimulation appeared to be higher when cells were electroporated with the former plasmid DNA. These data show that introduction of the LPS cDNA is capable of restoring LPS responsiveness in hyporesponsive C3H/HeJ cells.

TABLE 2. LPS stimulation of electroporated splenic B cells from responder C3H/HeOuJ mice or from C3H/HeJ mice[a]

DNA	Electroporation	Stimulation	Total nos. of plaques	
			C3H/HeOuJ	C3H/HeJ
None	−	None	15	12
None	−	LPS	151	10
None	−	EP	TMTC	TMTC
pCD-LPS	+	None	25	8
pCD-LPS	+	LPS	84	37
LB2.5	+	LPS	50	6

[a] Experimental details were the same as described in the footnote to Table 1. This experiment was performed three times and similar results were obtained. −, no electroporation performed; +, electroporation performed; TMTC, too many to count.

The cDNA sequence was analyzed by using a Genetics Computer Group program (6) and found to contain an open reading frame of 648 bp, which predicts a protein of 216 amino acids with a molecular weight of 24,423 kDa (Fig. 4A). At the amino acid level, *LPS* is identical to the Ran/TC4 GTPase found in humans and dogs, and it has 98.6 and 82.8% homology to the Ju93 and Spi1 proteins in chicken and yeasts, respectively (8, 9, 15, 30). At the DNA level, the homology of

A

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1    CCC CCC TCC GCG CGC CGG CGT CCG CTG CGT CTC CGG CAT TTG AAT CGC GTC   51
52   CGC CAT CTT TCC AGC TCC AGT CGG ACA GGC GCG GAG ACT CTT CTG GAA GGA  102

103  TCC GCC GCG ATG GCC GCC CAG GGA GAG CCG CAG GTC CAG TTC AAG CTC GTC  153
 1               M   A   A   Q   G   E   P   Q   V   Q   F   K   L   V    14

154  CTG GTG GGC GAC GGC GGC ACC GGG AAG ACA ACC TTC GTG AAG CGC CAC TTG  204
 15   L   V   G   D   G   G   T   G   K   T   T   F   V   K   R   H   L    31

205  ACG GGC GAG TTT GAG AAG AAG TAT GTA GCC ACC CTG GGC GTG GAG GTG CAC  256
 32   T   G   E   F   E   K   K   Y   V   A   T   L   G   V   E   V   H    49

256  CCG CTC GTC TTC CAT ACC AAC AGA GGA CCC ATC AAG TTC AAC GTG TGG GAC  306
 49   P   L   V   F   H   T   N   R   G   P   I   K   F   N   V   W   D    65

307  ACG GCC GGC CAG GAG AAG TTC GGG GGC CTG CGC GAT GGC TAC TAC ATC CAA  357
 66   T   A   G   Q   E   K   F   G   G   L   R   D   G   Y   Y   I   Q    82

358  GCC CAG TGT GCC ATT ATA ATG TTT GAT GTA ACC TCA AGA GTT ACT TAC AAG  408
 83   A   Q   C   A   I   I   M   F   D   V   T   S   R   V   T   Y   K    99

409  AAT GTA CCT AAC TGG CAT AGA GAT CTG GTA CGA GTG TGT GAA AAC ATC CCC  459
100   N   V   P   N   W   H   R   D   L   V   R   V   C   E   N   I   P   116

460  ATT GTA TTG TGT GGC AAC AAA GTG GAT ATT AAA GAC AGG AAA GTG AAG GCA  510
117   I   V   L   C   G   N   K   V   D   I   K   D   R   K   V   K   A   133

511  AAA TCT ATT GTC TTC CAC CGG AAG AAG AAT CTT CAG TAC TAT GAC ATT TCT  561
134   K   S   I   V   F   H   R   K   K   N   L   Q   Y   Y   D   I   S   150

562  GCC AAA AGT AAC TAC AAC TTT GAA AAG CCT TTC CTC TGG CTT GCC AGA AAG  612
151   A   K   S   N   Y   N   F   E   K   P   F   L   W   L   A   R   K   167

613  CTC ATT GGA GAT CCT AAC TTG GAG TTT GTT GCC ATG CCT GCT CTT GCC CCA  663
168   L   I   G   D   P   N   L   E   F   V   A   M   P   A   L   A   P   184

664  CCT GAG GTG GTC ATG GAC CCA GCT TTG GCA GCA CAG TAC GAG CAT GAT TTA  714
185   P   E   V   V   M   D   P   A   L   A   A   Q   Y   E   H   D   L   201

715  GAG GTT GCT CAG ACG ACT GCT CTC CCA GAT GAG GAT GAT GAC CTG TGA GAA  765
202   E   V   A   Q   T   T   A   L   P   D   E   D   D   D   L  term      216

766  AGT GAA GCT GGA TGC CCT GCG TCA GAA GTC TAG TTT TAT AGG CAA CTG TCC  816
817  TGT GAT GTC AAG CGG TGC AGC GCG TGT GCC ACC TTA TTT AGC TAA GCA GAT  867
868  CGT GTA CTT CAT TGG GAT GCT GAA GGA GAT GAA TGG GCT TCG AGT GAA TGT  918
919  GGC AGT TAA ACA TAC CTT CAT TTT TTG GAC TTG CAT ATT TAG CTG TTT GGA  969
970  ACA GAG TTG TTT CTT TTC TGA ATT TCA AAG ATA AGA CTG CTG CAG TCC CAT 1020
1021 CGC AAT ATC CAG TGG GGA AAT CTT GTT TGT TAC TGT CAT TCC CAT TCT TTT 1071
1072 CGT TAG AAT CAG AAT AAA GTT GTA TTT CAA ATA ATC TAA AAA AAA AAA AAA 1122
1123 AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AA          1166
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B

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                                         G1                       G2
MURINE   LPSn     MAAQGEPQVQFKLVLVGDGGTGKTTFVKRHLTGEFEKKYVATLGVEVHPLVFHTNRGPIK
HUMAN    Ran/TC4  ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
DOG      Ran/TC4  ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
CHICKEN  Ju93     ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
YEAST    Spi1     ::-:PQNVPT:::::::::::::::::::::::::::::I::::::::::H::::F:E:C

                       G3
MURINE   LPSn     FNVWDTAGQEKFGGLRDGYYIQAQCAIIMFDVTSRVTYKNVPNWHRDLVRVCENIPIVLC
HUMAN    Ran/TC4  ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
DOG      Ran/TC4  ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
CHICKEN  Ju93     ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
YEAST    Spi1     :::::::::::L::::::::::GQ:G:::::::::I::::::H:W:::::::::::::::

                   G4                         G5
MURINE   LPSn     GNKVDIKDRKVKAKSIVFHRKKNLQYYDISAKSNYNFEKPFLWLARKLIGDPNLEFVAMP
HUMAN    Ran/TC4  ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
DOG      Ran/TC4  ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
CHICKEN  Ju93     ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
YEAST    Spi1     :::::V:E::::::A:T:::::::::::::::::::::::::::::::V:N:::::::S:

MURINE   LPSn     ALAPPEVVMDPALAAQYEHDLEVAQTTALPDEDDDL
HUMAN    Ran/TC4  ::::::::::::::::::::::::::::::::::::
DOG      Ran/TC4  ::::::::::::::::::::::::::::::::::::
CHICKEN  Ju93     ::::::::::::::::::Q::QI:::::::::::::
YEAST    Spi1     :::::::QV:QQ:L:::QQEMNE:AAMP::::::::
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FIG. 4. (A) The nucleotide sequence of *LPS* cDNA and its deduced amino acid sequence. (B) Comparison of the deduced amino acid sequences of *LPS*, human Ran/TC4 (GenBank accession number M31469), dog Ran/TC4 (Z11922, S46784), chicken Ju93 (X66906), and yeast Spi1 (typed in from published sequence in reference 15). The complete sequence predicted for *LPS* is shown on the top line by the single letter amino acid code. Dots indicate identical residues to those of *LPS*; letters below the top line are different amino acid residues in the corresponding positions; the five boxes shown are the conserved functional domains shown to be involved in the binding and hydrolysis of guanine nucleotides (1, 7, 14, 20).

LPS to human Ran/TC4, dog Ran/TC4, chicken Ju93, and yeast Spi1 is 89.7, 90.7, 85.4, and 68.7%, respectively (8, 9, 15, 30). Therefore, this molecule is highly conserved throughout evolution, particularly in the five domains of the molecule, G1 to G5 (Fig. 4B), which have been shown to be involved in the binding and hydrolysis of guanine nucleotides (1, 7, 14, 20).

DISCUSSION

The Ran/TC4/Ju93/Spi1 proteins are a unique family of molecules among various types of GTP-binding proteins in that they are localized in the nucleus (15, 21), and they lack a consensus sequence such as the CAAX for *Ras* (13) and *Rho* and the CXC or CC for *Rab* (12, 33) in their carboxyl terminus. This consensus sequence is normally present on those GTP proteins that are membrane associated; instead, *LPS* has an acidic carboxyl terminal sequence, similar to other members in the Ran/TC4 family (3, 21). Functionally, Ran/TC4/Spi1 molecules are known to regulate cell-cycle progression (15, 16) and mRNA transport (2). They have been shown to act as a GTPase switch (16), which can prevent the premature initiation of mitosis when the Ran/TC4 is associated with a protein called RCC1 exchange factor (18, 31), or which can enhance their GTP hydrolysis activity when they are associated with a GTPase-activating protein.

In recent years, evidence has accumulated in the literature that different LPS-binding proteins on the cell membranes of responder cells may play a role as receptors (32). On the other hand, LPS has been shown to stimulate protein kinases in murine B cells and, most interestingly, pertussis toxin, which inactivates certain G proteins and inhibits LPS-induced B-cell DNA synthesis (10). Therefore, our finding that the Ran/TC4 G protein is the expression product of the *LPS* gene is at least consistent with what little is known about the LPS signalling mechanism. Indeed, the various functions of Ran/TC4 in regulating cell-cycling progression and nuclear transport are also consistent with a number of defects observed in C3H/HeJ mice, such as the fact that C3H/HeJ B cells do not proliferate or differentiate when exposed to LPS. In addition, the activation of C3H/HeJ macrophages by LPS to produce various cytokines, including tumor necrosis factor and interleukin-1 (the overproduction of which in responder animals is believed to contribute to endotoxemia), is impaired.

By using a modified plaque assay, we have isolated a cDNA whose expression in C3H/HeJ defective splenic B cells resulted in rendering the cells responsive to LPS stimulation. Recently, we have also shown that when the *LPS* gene is electroporated into an LPS nonresponsive macrophage cell line, GG2EE (4), its expression in these cells restored their ability to respond to LPS challenge (data not shown). By in situ hybridization, the gene has been located to chromosome 4, although the exact location could not be accurately determined at this time (data not shown). We have also isolated four independent clones of the *LPS* cDNA from C3H/HeJ splenic B cells; sequence analysis of all four clones of the cDNA indicated the presence of a specific point mutation at position 870, where there is a single base substitution from T to C (see Fig. 4). A more complete presentation of this work is in preparation. Interestingly, this single point mutation occurs at the 3′-untranslated region, and by Northern (RNA) blot analysis, there is no significant difference between C3H/HeJ and C3H/HeOuJ cells at the level of mRNA for this gene (data not shown). Given that this gene encodes for a Ran GTPase, which is known to play important roles in nuclear transport, cell cycle, and differentiation (18, 21, 31, 33), the nature of this point mutation in C3H/HeJ cells may be related to its intracellular distribution. Improper intracellular distribution in C3H/HeJ cells may result in inappropriate nuclear transport or cell cycling, thereby accounting for the LPS hyporesponsiveness of C3H/HeJ cells. In summary, we have isolated an LPS-responsive gene and are in a position to determine whether the various immunostimulating and pathophysiological responses to LPS characteristic of responder animals and deficient nonresponder animals are controlled by this gene and its product. In so doing, we should be able to develop a better understanding of the mechanism of action of LPS in activating host cells and possibly of how to turn it off.

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