Bacterial Lipopolysaccharide Induces Expression of the Stress Response Genes hop and H411*

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CD14-transfected Chinese hamster ovary K1 fibroblasts (CHO/CD14) respond to lipopolysaccharide (LPS) by metabolizing arachidonic acid and with translocation of NF-κB to the nucleus. Although previous experiments failed to identify the production of tumor necrosis factor-α and interleukin (IL)-1β by CHO/CD14 cells, LPS did induce the expression of IL-6 mRNA and the subsequent release of the IL-6 protein. To identify additional LPS-inducible genes, a cDNA library derived from LPS-stimulated CHO/CD14 cells was screened by subtractive hybridization. Fourteen genes were found to be expressed differentially, and two were analyzed in detail: hop (Hsp70/Hsp90-organizing protein), which is the hamster homologue of the stress-inducible yeast gene, STI1, and clone H411, which encodes a novel LPS-inducible growth factor. In response to LPS, the expression of Hop mRNA was also increased in both the murine macrophage cell line, RAW 264.7, as well as in primary hamster macrophages. This suggested that the up-regulation of Hop expression is part of the macrophage stress response to LPS. Clone H411 encodes a protein in the epidermal growth factor-like repeat protein family. Overexpression of H411 cDNA in the RAW 264.7 macrophage cell line promoted an increased growth rate, suggesting that expression of H411 is part of the proliferative cell response to LPS. Both Hop and H411 represent novel gene products not previously recognized as part of the complex biological response to endotoxin.

Lipopolysaccharide (LPS, endotoxin), the major structural component of the outer leaflet of Gram-negative bacteria, is thought to initiate the development of Gram-negative sepsis (1, 2). The interaction of human mononuclear phagocytes with bacterial lipopolysaccharide leads to a dramatic change in gene expression in immune cells. A large number of genes have been identified in LPS-challenged animals (3–5), most of which protect the host from invading pathogens. Paradoxically, the excessive production of these gene products, such as the proinflammatory cytokines (6–8), may eventually cause the septic shock syndrome. Thus, bacterial invasion and the subsequent proinflammatory response represent a major survival challenge for the host.

Most studies of the biological response to infection have focused on the mononuclear phagocyte as a mediator of tissue damage. These cells express CD14, a major LPS receptor, on their surface (9). A great amount of data supports the concept that mononuclear phagocytes have a special role in the pathophysiology of endotoxin-induced inflammation resulting from LPS-inducible cytokine production. Other cell types, including cells of mesenchymal origin such as epithelium, fibroblasts, and endothelium, also serve as targets for released bacterial products. To date, the data suggest that the majority of these cells use a proteolytic fragment of CD14 as a soluble LPS receptor (10).

Chinese hamster ovary (CHO)-K1 fibroblasts are a well-characterized cell line (11) which become exquisitely sensitive to LPS, when CD14 is expressed following transfection (CHO/CD14) (12). CHO/CD14 cells mimic many of the responses observed in natural target cells. These responses include the inducible release of arachidonic acid metabolites (12) and translocation of the transcription factor nuclear factor-κB (NF-κB) (13). LPS-induced cellular activation represents a strong stress signal for cells. Among the various proteins that help cells respond to stress is a family referred to as “heat shock proteins” (Hsp). First described as being up-regulated in response to hyperthermia (14), heat shock proteins are also induced by other environmental stressors, including UV light, mechanical trauma, or exposure to a variety of pathogens (15). Heat shock proteins are thought to function as protein chaperones (16) that act by stabilizing intermediate polypeptides during folding, assembly, and disassembly.

Protein maturation requires the highly organized machinery of chaperones. A well described example is the assembly of the progesterone receptor, which involves at least eight different proteins (17), including Hsp70 and Hsp90, p48, and an organizing protein. This organizing protein, formerly known as p60, has recently been renamed Hop (Hsp70/Hsp90-organizing protein) because of its apparent function in human cells (18). Hop appears to be the human homologue of the yeast stress-inducible protein 1 (STI1) (19), first described by Nicolet and Craig (20). Although STI1 is apparently not essential for cellular survival and growth under normal conditions, null mutants of STI1 have reduced target protein activity (21) and survive poorly after thermal shock (20).

In addition to intracellular responses to external stimuli, cells may release substances that result in extracellular remodeling. The inflammatory potential of such substances may be quite different from cytokines. The type of environmental signal encoded by an extracellular growth factor, for example, may be more important for development and/or repair than for...
microbicidal activity. The extracellular matrix appears to be especially rich in proteins. Many of these proteins such as fibrillin (22), Notch of Drosophila (23), or transforming growth factor-β1-binding protein (24) contain epidermal growth factor (EGF)-like domains that are crucial for proper function of the proteins (25–28). These domains consist of motifs of 35–40 amino acids, with a conserved spacing of six cysteine residues, as was first described for EGF (29). Proteins with EGF-like domains are often implicated in cell growth and differentiation (30).

We report here that LPS induces the production of the proinflammatory cytokine, IL-6, from CD14-bearing CHO cells, similar to responses observed in primary phagocyte cultures (31–33) and many other LPS-responsive cells, including native fibroblasts (34–37). In addition, when LPS-inducible gene expression was examined by the analysis of a subtractive hybridization, a number of novel genes were found to be induced. These include the STT1 homologue hop and an EGF-like protein that we have designated as “H411.” The biologic properties of these gene products suggest that they participate in the repair process that would be necessary after a septic insult.

**EXPERIMENTAL PROCEDURES**

**Reagents**—Unless otherwise stated, reagents were purchased from Sigma. All solutions used for tissue culture were provided as “pyrogen-free” by the manufacturer. Dulbecco’s modified Eagle’s medium, Ham’s F-12 medium with 1-glutamine, pyrogen-free water, and PBS were obtained from BioWhittaker (Walkersville, MD). Fetal bovine serum was purchased from Life Technologies, Inc. ReLPS was the gift of Dr. Nilofer Ali (University of Cincinnati, Cincinnati, OH) were isolated.

**Table I. Primer specifications for RT-PCR**

| Primer                  | Sequence (5′–3′)                                      | Annealing temperature | Size  | Cycles |
|-------------------------|-------------------------------------------------------|-----------------------|-------|--------|
| IL-6 sense (mouse)      | GAAACACATTCTGAACTTCCAGGTGGAAAGCAGAT                  | 56                    | 1350  | 15     |
| IL-6 antisense (mouse)  | TTCTGAGTTGATCTTATGTTGTTTTCGGATTGTCG                 | 55                    | 184   | 15     |
| IL-6 sense (hamster)    | AGCTTTGTGACATTGTTGTTTTCGGATTGTCG                     | 60                    | 443   | 15     |
| IL-6 antisense (hamster)| GATGCTTGTCTTGGACCACTTGGTCTTCTTG                    | 59                    | 537   | 15     |
| GAPDH sense             | TTACCCCAAGGCATTGAGCACT                              | 59                    | 537   | 15     |
| GAPDH antisense         | GGGAGAAGGAAAGGGGGAAGAAAGA                           | 57                    | 727   | 15     |
| Hop sense               | CACGAGGGATCACGCAATGCA                                 | 57                    | 431   | 15     |
| Hop antisense           | AGGGAGAAGGAAAGGGGGAAGAAAGA                           | 57                    | 431   | 15     |
| Hsp70 sense             | GCGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG | 59                    | 537   | 15     |
| Hsp90 sense             | GGCAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG | 59                    | 537   | 15     |
| Hsp90 antisense         | GGCAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG | 59                    | 537   | 15     |

**Generation of the cDNA Library**—A cDNA library was constructed from CHO/CD14 cells using the ZAP Express cDNA Gigapack III Gold cloning kit (Stratagene, La Jolla, CA) per the manufacturer’s instructions. Briefly, CHO/CD14 cells were exposed to 100 ng of LPS/ml. The resulting 354-bp fragment was subsequently subcloned (TA cloning kit, Invitrogen, Carlsbad, CA) and sequenced at the Boston University Core Facility using an Applied Biosystems Inc. 3137A automated sequencer (Applied Biosystems Inc., Foster City, CA). Table I shows the sequence of all primers, the annealing temperature, the size of the PCR product, and the number of cycles used in the PCR reaction.

**Hamster-specific PCR primers were developed using the sequence of the hamster IL-6 fragment which we cloned using murine IL-6 primers (Table I) and total RNA from stimulated CHO/CD14 (1 h, 100 ng of ReLPS/ml). The resulting 354-bp fragment was subsequently subcloned (TA cloning kit, Invitrogen, Carlsbad, CA) and sequenced at the Boston University Core Facility using an Applied Biosystems Inc. 3137A automated sequencer (Applied Biosystems Inc., Foster City, CA).**

**Screening of the IL-6 Activity**—Cells were seeded at 1 × 10^6 cells/ml in CM and incubated overnight before addition of LPS. Supernatants were collected after 24 h and bioassayed for IL-6 activity using the B-9 hybridoma cell line as described (38).

**Reverse Transcription-PCR**—Total RNA from CHO/CD14 cells or Chinese hamster peritoneal macrophages was harvested using TriReagent (Molecular Research Center, Cincinnati, OH) according to manufacturer’s protocol. One to two μg of total RNA was reverse-transcribed in a volume of 20 μl using Superscript II reverse transcriptase according to the manufacturer’s protocol (Life Technologies, Inc.). Two μl of the resulting cDNA was used in a 25-μl PCR reaction as described (39). The PCR was conducted in an automatic thermal cycler (Hybaid, Franklin, MA). Table I shows the sequence of all primers, the annealing temperature, the size of the PCR product, and the number of cycles used in the PCR reaction.

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**Generation of the cDNA Library**—A cDNA library was constructed from CHO/CD14 cells using the ZAP Express cDNA Gigapack III Gold cloning kit (Stratagene, La Jolla, CA) per the manufacturer’s instructions. Briefly, CHO/CD14 cells were exposed to 100 ng of LPS/ml. After 4 h of stimulation, total RNA was extracted with TriReagent. Messenger RNA was purified from total RNA using the PolyATtract mRNA isolation system (Promega, Madison, WI). Five μg of poly(A)^+ mRNA were used as starting material for the library. The primary 4-h library was titered and found to contain 3.5 × 10^6 individual clones. The library was amplified on solid medium and assessed for quality by Southern hybridization. The percentage of GAPDH-encoding clones was approximately 0.05%, as determined by probing phageplits on nitrocellulose with ^32P-labeled GAPDH probe.

**Screening of the cDNA library with a Subtracted cDNA Probe**—To identify novel LPS-induced genes, we generated subtracted cDNA from CHO/CD14 cells that had been stimulated for 4 h with 100 ng of LPS/ml using the Subtractor Kit (Invitrogen), closely following the manufacturer’s instructions. Briefly, mRNA from stimulated CHO/CD14 cells was purified and used to generate cDNA; contaminating mRNA was removed by alkali treatment. RNA from unstimulated CHO/CD14 cells was photobiotinylated, and 10 μg was hybridized with 1 μg of stimulated cDNA for 66 h at 42 °C. RNA/cDNA hybrids derived from both the unstimulated photobiotinylated RNA pool and the stimulated cDNA pool were then removed by adding streptavidin followed by phenol/ chloroform extraction. The remaining cDNA was used as a template to generate random primed ^32P-labeled DNA probe (40).

**Screening of the cDNA library for induced genes was performed with the subtracted probe. Thirty-five thousand plaque-forming units/plate**
were transferred onto nylon membranes (Hybond, Amersham Pharmacia Biotech), dried, and dehydrated at 42 °C overnight. Membranes were hybridized with the labeled DNA probe (~1 × 10⁶ cpm/ml) at 65 °C overnight, washed 3 times, and subjected to autoradiography (Kodak X-AR, Eastman-Kodak Co.) at ~80 °C with intensifying screen (NEN Life Science Products). Positive clones were localized and removed from the original plates using the wide end of Pasteur pipettes. The phages were re-titered and used for the second round of screening. Nineteen isolated positive clones were subsequently converted into phagemids by single clone excision. Co-infection of the *Escherichia coli* XL1-Blue MRF’ cells with a positive phage and a helper phage led to the excision of the phagemid vector including the original cDNA insert packaged as filamentous phage particles. After infection of the susceptible bacterial strain XLORL, these were converted into plasmids. Restriction enzyme analysis revealed that 14 of these cDNA clones appeared to represent unique clones. Sequence assembly was performed using the SEQMAN II module of Lasergene biocomputing software (DNASTAR, Madison, WI). Additional gene analysis and sequence alignment was performed using Genelnasator software (Textco, West Lebanon, NH).

Transfection of RAW 264.7 Cells—The phagemids, pBK-CMV-411 and pBK-CMV-neo (no insert), were obtained by single clone excision. RAW 264.7 (1 × 10⁶ cells) were plated in a 10-cm tissue culture dish (Falcon) and, after overnight adhesion, were transfected with either 10 μg of pBK-CMV-411 or pBK-CMV-neo using the calcium-phosphate precipitation method (41). Stable transfectants were selected with one mg/ml G418 and analyzed after cell death had ceased and they had returned to logarithmic growth conditions.

Growth Rate of RAW 264.7 Cells—RAW 264.7 cells, stably transfected with pBK-CMV phagemids containing either cDNA for clone 411 or the mock vector insert, were plated in complete medium at a density of 2 × 10⁵ cells/well in a 12-well tissue culture plate (Costar). After 4 h, they were transferred to the original plates using the wide end of Pasteur pipette. Twenty-four hours later, plates were counted using a hemocytometer. All experiments were repeated using four individual transfections and yielded nearly identical results.

RESULTS

LPS Induces Increased Expression of IL-6 mRNA in CHO/CD14 Cells—We previously observed that LPS induces NF-κB translocation in CHO/CD14 cells (13). To investigate if CHO/CD14 cells were also capable of producing proinflammatory cytokines, we examined IL-6 mRNA expression in CHO/CD14 cells after stimulation with LPS. Hamster-specific IL-6 primers were designed only after we subcloned a hamster cDNA fragment of 303 bp using murine IL-6 primer (~54% of the mature murine IL-6 peptide). Sequence comparison of this fragment (GenBank™ accession number AF044667) revealed ~93% identities to murine and rat IL-6 at the cDNA level and 69 and 76%, respectively, at the protein level (Fig. 1). We determined the expression of steady-state IL-6 mRNA levels following the stimulation of CHO/CD14 cells with LPS by using reverse transcriptase PCR (Fig. 2). Unstimulated CHO/CD14 cells expressed low but detectable levels of IL-6 mRNA, but this expression was significantly up-regulated after 1–2 h of exposure to LPS. After 8 h, IL-6 mRNA expression returned to basal level.

Consequently, we tested if CHO/CD14 cells were also able release IL-6 protein following the stimulation with LPS. We compared the LPS-induced IL-6 release of CHO/CD14 cells with CHO cells transfected with the empty, neomycin-confering vector (CHO/neo). LPS concentrations as low as 10 ng/ml induced secretion of ~1450 pg/ml IL-6, whereas the release of IL-6 in CHO/neo cells was only minimally increased over basal IL-6 levels (Fig. 3).

LPS-induced Up-regulation of Hop mRNA—To identify novel LPS-inducible genes, we screened a CDNA library derived from CHO/CD14 cells stimulated for 4 h with LPS. We used cDNA generated by the subtraction of unstimulated CHO/CD14 cell mRNA from LPS-induced cDNA as a radiolabeled probe for differential expression genes. After three rounds of screening, we obtained 14 distinct clones, shown by comparison of different restriction enzyme digestion patterns (data not shown). Sequence analysis of a single sequence run on these clones revealed hamster homologues to six known mammalian genes and eight novel genes (data not shown).

The first gene we cloned was identified as the hamster homologue of *hop* (GenBank™ accession number AF039202), which encodes a protein homologous to the stress-inducible yeast protein, STI1 (20). STI1 protein is highly conserved among eukaryotic species. At the cDNA level, the hamster sequence shares 94, 93, and 89% identities to rat, murine, and human *hop* sequences, respectively. It also shares 54% identity to the nucleotide sequence of yeast STI1 (data not shown). At the protein level, sequences from hamster, rat, mouse, and human show an even higher degree of similarity (about 97%), whereas hamster Hop and yeast STI1 share 42% identical amino acids (Fig. 4).

We then confirmed by semi-quantitative RT-PCR that LPS induces the up-regulation of Hop mRNA not only in CHO/CD14 cells (Fig. 5B) but also in primary cultures of Chinese hamster peritoneal macrophages (Fig. 5A). RT-PCR results were confirmed by performing Northern blot analysis of LPS-treated RAW-264.7 cells (data not shown). After 4 h of stimulation with LPS, mRNA expression of Hop was consistently up-regulated in both cell types. We were also interested to determine if Hop and other Heat shock proteins were up-regulated simultaneously, since Hop has recently been shown to organize complexes of Hsp70 and Hsp90 (42). Over the same time course, Hsp70 was even more strongly up-regulated than hop or Hsp90 (Fig. 5B).
LPS-induced Up-regulation of Hamster H411—The second clone that we investigated further was designated H411 (Gen-Bank™ accession number AF046870). After complete sequencing of the cDNA, we designed specific PCR primers for this clone. We observed induction of H411 mRNA in CHO/CD14 cells as early as 1 h after stimulation with LPS. H411 expression continued to increase over the 4-h stimulation period (Fig. 6B). The same induction pattern could be seen in peritoneal macrophages from Chinese hamsters (Fig. 6A). The complete sequence of the isolated cDNA encoding for H411 includes one large open reading frame (bp 235–1623) that contains at least two potential translation start sites at 235 and 292. The second ATG is more likely to be the true initiation codon since it starts with a 27-amino acid signal sequence with a cleavage site between Pro-27 and Gln-282 (43). H411 contains six EGF-like repeats, sequences that are common to a broad family of proteins involved in cell growth and differentiation (e.g., fibrin(44), fibrillin(22), notch of Drosophila melanogaster (23)). The 3’-untranslated region contains a single polyadenylation site.

Comparison of the amino acid sequence of H411 with Gen-Bank™ entries using the NCBI Blast Search program revealed similarities (56% identity at the amino acid level) to the sequence of human extracellular protein S1–5 (Fig. 7). Human extracellular protein S1–5 has been shown up-regulated in both the senescent and quiescent human fibroblasts from a patient with Werner’s syndrome of early aging (45). Recently, the sequence of a protein named HCABA58X, a putative extracellular-epidermal growth factor, was reported in a proprietary patent data base (accession number 32110 in the GENESEQ database, Derwent Scientific Publications and The Oxford Molecular Group). Sequence comparison of HCABA58X with H411 demonstrated 96% identity (Fig. 7), suggesting that HCABA58X represents the human homologue of H411.

Both the HCABA58X (patent application) and S1–5 (45) were found to be involved in cell growth. Thus, we sought to determine if overexpression of H411 mRNA altered the growth characteristics of cells. We transfected the murine macrophage cell line, RAW 264.7, with a mammalian expression vector that contains H411 or with an empty vector. After selection of stably transfected cells in G418, cells were plated in 12-well dishes, and viable cells were counted from triplicate wells each from four successive days. We observed that these bulk cells, which overexpress H411, showed a significantly higher growth rate compared with cells transfected with the cDNA for the empty vector only (Fig. 8). This result was consistently observed in four independent transfections.

DISCUSSION

The biological responses to LPS are remarkably complex, not surprising considering the pleiotropic effects of this important bacterial product. We chose to examine LPS-induced events using genetic techniques in CD14-transfected CHO fibroblasts in a broad effort to dissect signal transduction events. The expression of CD14 renders CHO fibroblasts responsive to picogram per ml concentrations of LPS (12). Many, if not all, of the observed events appear to reflect an orchestrated cellular response to bacterial infection. LPS induces the translocation of NF-xB and the release of arachidonic acid in a myriad of immune and non-immune target cells. These important inflammatory events have also been observed in CHO/CD14 cells. However, prior to this report, there were no data suggesting that LPS could induce the production of cytokines in CHO/CD14 cells, an important response to LPS by primary target cells. In theory, this could potentially limit the usefulness of this cell line as a genetic model of LPS responsiveness.

CHO/CD14 cells have been studied as a model of both phagocytic and non-phagocytic cells because they appear to contain many elements of the LPS signal transduction apparatus. Although investigators have traditionally focused on macrophages, virtually every cell type in the body responds to endotoxin, a fact that may ultimately prove to be important in host defense. Human gingival fibroblasts have been reported capable of responding to LPS by producing IL-6 (37), whereas fibroblasts of lung tissue apparently do not respond to endotoxin (46). Therefore, in the absence of membrane CD14 expression, it seems that the tissue origin somehow determines the responsiveness of a given cell type to LPS. To date, the reason for this differential behavior has not been clarified. Several groups (10, 13) have reported that LPS-induced responses observed in non-CD14 bearing cells are dramatically enhanced by the presence of soluble CD14, but we have not observed significant sCD14-mediated responses to LPS in non-transfected CHO cells. We propose that the differential expression of IL-6 observed in LPS-responder gingival fibroblasts and LPS non-responder lung fibroblasts is due to differential expression of a CD14-associated co-receptor. The recent description of TLR2 (47, 48) and TLR4 (49) as potential CD14-associated signal transducers, and the derivation of mutant LPS non-responder cell lines (50), may allow this hypothesis to be tested directly in the near future.

Among the best studied heat shock proteins are Hsp70 and Hsp90. LPS is an inducer of Hsp70 expression in monocytes/macrophages (51, 52), and overexpression of Hsp70 (53, 54) or hyperthermia (55) conferred protection against endotoxin and endotoxin-mediated effects. In addition, Feinstein et al. (56) observed that overexpression of Hsp70 limited LPS-induced nuclear localization of the NF-xB p65 subunit. The proper functioning of Hsp70 requires a cycle of ATP binding, hydrolysis, and ADP exchange for ATP (reviewed in Ref. 57), a biological role that is thought to be played by Hop. Hop, first described as a stress-related protein that interacts with Hsp70 and Hsp90 (58), is now believed to organize a complex of these heat shock proteins (42). Gross and Hessefort (59) observed that a Hop homologue in rabbit catalyzes the exchange of Hsp70-bound ADP to ATP, supporting the concept that Hop is necessary for the proper functioning of Hsp70. It appears that the LPS-induced up-regulation of Hsp70 is a counter-regula-

![Fig. 3. LPS-induced release of IL-6. CHO/CD14 (black bars) or CHO/neo cells (open bars) were seeded at 1 × 10⁵ cells/ml and incubated overnight before addition of indicated amounts of LPS. Supernatants were collected after 24 h and assayed for IL-6 bioactivity (38). Values are means ± S.D. of one out of three independent experiments.](image-url)
LPS-induced Up-regulation of hop and H411

The response of the cell that confers protection against LPS-mediated events. Simultaneous up-regulation of Hop and Hsp90 should confer a survival advantage to LPS-stressed cells because at least a portion of the total pool of Hsp70 operates as complexes with Hsp90. Our data support this hypothesis.

Many proteins of the extracellular matrix (60–62) contain EGF-like domains and have a growth promoting activity. LPS is a classic mitogen in B-lymphocytes (63), although the mechanism of this action remains unclear. In this report we describe that LPS induces the up-regulation of an apparently extracellular protein, H411, which contains EGF-like repeats and promotes growth. Microinjection of mRNA of a highly homologous protein, S1–5 (45), led to an autocrine/paracrine stimulation of DNA synthesis. H411 and S1–5 are about 56% similar at the amino acid level and, along with HCABA58X, are likely to be members of the same protein family. Recent studies using a rat homologue of S1–5 suggested that the growth-promoting effects of this protein resulted from complex formation with a growth-suppressing protein (64). By analogy, a similar mechanism of action might be predicted for H411. The biological significance of LPS-induced H411 production is unknown, but one might imagine that infected tissues require a proliferative fibroblast response as a part of a normal healing response to injury. On the other hand, the proliferative responses of fibroblasts to endotoxin might also prove to be deleterious to the host. Substances such as H411 and similar growth factors may play a role in common sequelae of septic shock, such as the acute respiratory distress syndrome, which often results in crippling or lethal pulmonary fibrosis.

Bacterial sepsis is thought to result from the interaction of bacterial products with host receptors, which subsequently leads to the activation of the inflammatory response. Most of the attention has focused on the induction of proinflammatory mediators, such as cytokines, and the role these molecules play in the hypotensive shock state. Yet, as shown by these findings, the response to bacterial products is broader than was previously recognized. The widely accepted model of the pathogenesis of sepsis is that the syndrome results from an invasive infection, which provokes an overly profuse cytokine response. Yet, this paradigm is clearly overly simplistic. A large number of gene products, such as heat shock proteins and growth factors, undoubtedly influence the outcome of septic insults.
sequence (GenBank™ accession number AF046870) was translated into protein sequence and aligned with the sequence of HCABA58X (see text for details) and human extracellular protein S1–5 (GenBank™ accession number U03877 (45)). Identical residues are shaded.

Growth rate of RAW 264.7 cells transfected with H411 cDNA. RAW 264.7 cells were transfected with phagemids coding for cDNA. Viable cells were counted on 3 consecutive days. Each point represents the mean number of cells ± S.D. The results are representative of one of four similar experiments using four independent transfections.

However, these have received virtually no experimental attention. A more comprehensive understanding of the true nature of the response to infection will not only result in therapeutic breakthroughs but should help avoid the problems that have thus far plagued experimental therapies for sepsis (65, 66).

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