The induction of macrophage gene expression by LPS predominantly utilizes Myd88-independent signaling cascades

Harry Bjorkbacka
Lund University

Katherine A. Fitzgerald
University of Massachusetts Medical School

Francois Huet
Harvard Medical School

See next page for additional authors
The induction of macrophage gene expression by LPS predominantly utilizes Myd88-independent signaling cascades

Authors
Harry Bjorkbacka, Katherine A. Fitzgerald, Francois Huet, Xiaoman Li, James A. Gregory, Melinda Lee, Christine M. Ordija, Nicole E. Dowley, Douglas T. Golenbock, and Mason W. Freeman

Rights and Permissions
Citation: Physiol Genomics. 2004 Nov 17;19(3):319-30. Epub 2004 Sep 14. Link to article on publisher’s site

This article is available at eScholarship@UMMS: http://escholarship.umassmed.edu/infdis_pp/95
The induction of macrophage gene expression by LPS predominantly utilizes Myd88-independent signaling cascades

Harry Björkbacka, Katherine A. Fitzgerald, François Huet, Xiaoman Li, James A. Gregory, Melinda A. Lee, Christine M. Ordija, Nicole E. Dowley, Douglas T. Golenbock and Mason W. Freeman


You might find this additional info useful...

This article cites 54 articles, 32 of which can be accessed free at:
http://physiolgenomics.physiology.org/content/19/3/319.full.html#ref-list-1

This article has been cited by 1 other HighWire hosted articles
Human UMP-CMP Kinase 2, a Novel Nucleoside Monophosphate Kinase Localized in Mitochondria
Yunjian Xu, Magnus Johansson and Anna Karlsson
[Abstract] [Full Text] [PDF]

Updated information and services including high resolution figures, can be found at:
http://physiolgenomics.physiology.org/content/19/3/319.full.html

Additional material and information about Physiological Genomics can be found at:
http://www.the-aps.org/publications/pg

This information is current as of March 28, 2011.

Physiological Genomics publishes results of a wide variety of studies from human and from informative model systems with techniques linking genes and pathways to physiology, from prokaryotes to eukaryotes. It is published quarterly in January, April, July, and October by the American Physiological Society, 9650 Rockville Pike, Bethesda MD 20814-3991. Copyright © 2005 by the American Physiological Society. ISSN: 1094-8341, ESSN: 1531-2267. Visit our website at http://www.the-aps.org/.
The induction of macrophage gene expression by LPS predominantly utilizes Myd88-independent signaling cascades

Harry Björkbacka,1,4 Katherine A. Fitzgerald,2 François Huet,1,4 Xiaoman Li,3 James A. Gregory,1 Melinda A. Lee,1 Christine M. Ordija,1 Nicole E. Dowley,2 Douglas T. Golenbock,2 and Mason W. Freeman1,4

1Lipid Metabolism Unit, Department of Molecular Biology, Massachusetts General Hospital, Boston; 2Division of Infectious Disease and Immunology, University of Massachusetts Medical School, Worcester; 3Department of Statistics, Harvard University, Cambridge; and 4Harvard Medical School, Boston, Massachusetts

Submitted 18 August 2004; accepted in final form 10 September 2004

The induction of macrophage gene expression by LPS predominantly utilizes Myd88-independent signaling cascades. Physiol Genomics 19: 319–330, 2004. First published September 14, 2004; doi:10.1152/physiolgenomics.00128.2004.—Myeloid differentiation protein-88 (MyD88) is a signal adaptor protein required for cytokine production following engagement of Toll-like receptors (TLRs) by their cognate ligands. Activation of both TLR-3 and TLR-4, however, can engage signaling events independent of MyD88 expression. The relative importance of these MyD88-dependent and -independent signaling pathways in the macrophage response to lipopolysaccharide (LPS) is unknown. Here we define these events using microarray expression profiling of LPS-stimulated macrophages taken from MyD88-null and wild-type mice. Of the 1,055 genes found to be LPS responsive, only 21.5% were dependent on MyD88 expression, with MyD88-independent genes constituting 74.7% of the genetic response. This MyD88-independent gene expression was predominantly transcriptionally regulated, as it was unaffected by cycloheximide blockade of new protein synthesis. A previously undescribed group of LPS-regulated genes (3.8%), whose induction or repression was significantly greater in the absence of MyD88, was also identified by these studies. The regulation of these genes suggested that MyD88 could serve as a molecular brake, constraining gene activity in a subset of LPS-responsive genes. The findings generated with LPS stimulation were recapitulated by exposure of macrophages to live Escherichia coli. These expression-profiling studies redefine the current dogma of TLR-4 signaling and establish that MyD88, although essential for some of the best-characterized macrophage responses to LPS, is not required for the regulation of the majority of genes engaged by macrophage exposure to endotoxin or live bacteria.

monocytes/macrophages; lipopolysaccharide; gene regulation; signal transduction; cellular activation

THE ACTIVATION OF THE INNATE immune response by infectious microorganisms requires the integration of complex networks of signal transduction pathways, leading to the induction of specific sets of genes (18). Toll-like receptors (TLRs) have emerged as a major recognition and signaling component of the mammalian host defense. TLRs recognize molecular products derived from all the major classes of microbes including bacteria, viruses, yeast, and fungi. Elucidating the cellular responses following the activation of individual TLRs is fundamental to our understanding of innate immunity.

TLR superfamily members share a conserved Toll/interleukin-1 resistance (TIR) domain, which is also present in the interleukin-1 receptor (IL1R) and related proteins, such as SIGIRR and ST2 (27, 48). Engagement of these receptors by their respective ligands allows the recruitment of one or more TIR domain-containing adapter molecules. These adapters, myeloid differentiation protein-88 (MyD88), Mal (also known as TIRAP), TRIF (also known as TICAM-1), or TRAM (also known as TRIP, TICAM2), provide a structural platform for the recruitment of kinases and downstream effector molecules (for a complete review see Refs. 31, 47). The complex signaling network initiated by the TIR domain-mediated interaction of TLRs and adapter proteins ultimately dictates the distinct pattern of gene expression that one observes in response to different TLR agonists.

MyD88, the first TIR-domain adapter molecule to be described, is essential for signaling by all IL1R/TLR family members with the exception of TLR-3 (15, 33). The recruitment of MyD88 to oligomerized TIR domains engages NF-κB and MAPK signaling cascades, resulting in the expression of proinflammatory cytokines, such as TNF (5, 19, 32). Consistent with these observations, the production of TNFα is impaired in MyD88-deficient macrophages following stimulation by various TLR ligands or IL-1β (1, 23). Although MyD88 initially appeared to be required for signal transduction events engaged by TLR activation, certain TLR agonists, such as lipopolysaccharide (LPS), have been shown to generate signals in the absence of MyD88. These responses include the upregulation of costimulatory molecules on dendritic cells (22), caspase-1-dependent release of IL-18 (38), and expression of type I interferons (IFN-β) and interferon-stimulated genes (24). To clarify the role of MyD88, we set out to determine the relative contribution of MyD88-dependent and -independent signaling events in the totality of macrophage responses to endotoxin or live Escherichia coli. To accomplish this, we conducted genome-wide expression profiling experiments in macrophages taken from MyD88-null and wild-type (WT) mice. Surprisingly, we found that the preponderance of LPS or E. coli-responsive genes was regulated by the MyD88-independent signaling network. Although the MyD88-independent genes include many that are known to be important mediators of innate immunity, a large group of genes in this class are of unknown function. These findings indicate that recently identified adaptor molecules, other than MyD88, that lie downstream of TLR-4 play a more important role in the response to...
bacterial engagement by macrophages than was previously recognized.

**EXPERIMENTAL PROCEDURES**

**Materials.** MyD88-deficient mice were a gift from S. Akira (Osaka, Japan) (1) and were backcrossed onto a C57BL/6 background for a minimum of six generations, theoretically yielding mice that are genetically \(~98.5\%\) C57BL/6. LPS derived from *E. coli* strain O111:B4 was purchased from Sigma, dissolved in deoxycholate, and re-extracted with phenol:chloroform as described (14). *E. coli* O111:B4 (smooth strain) gram-negative bacteria were purchased from American Type Culture Collection (ATCC, Manassas, VA). Endotoxin-free polycarbonate filters were from Amersham Pharmacia (Piscataway, NJ) and was used in the absence of lipid reagents. Sendai virus was from Charles River Laboratories (Wilmington, MA). Malp-2 was from EMC Microcollections (Tuebingen, Germany). R-848 was from GL Synthesis (Worcester, MA). CpG DNA 1668 ODN was from MWG Biotech, and cycloheximide was from Sigma (St. Louis, MO). The plasmids, pCDNA3-MyD88-AU1, pEF-Bos-Trif-Flag, and NF-kB-luciferase, used in cell transfection experiments have been previously described (10). IRF3–5D was from John Hiscott (Montreal, Quebec, Canada). p65-HA was from Tom Maniatis (Harvard, MA). The human Vigi1 promoter (450 bp) was cloned from Thp-1 genomic DNA into pGL-3 enhancer (Promega, Madison, WI).

**Cell culture and RNA isolation.** Mice were anesthetized with isoflurane and then killed by cervical dislocation. Bone marrow was isolated from 6- to 8-wk-old C57BL/6 WT and MyD88-deficient (a source for colony-stimulating factor (CSF)-1) for 8 days. Embryonic fibroblasts were derived from WT or MyD88-deficient mice. Macrophages from these marrows were cultured in DMEM media supplemented with 10% FBS and 15% L929 conditioned media and antibiotics. Thioglycolate-elicited peritoneal-exudate macrophages (Worcester, MA). CpG DNA 1668 ODN was from MWG Biotech, and cycloheximide was from Sigma (St. Louis, MO). The plasmids, pCDNA3-MyD88-AU1, pEF-Bos-Trif-Flag, and NF-kB-luciferase, used in cell transfection experiments have been previously described (10). IRF3–5D was from John Hiscott (Montreal, Quebec, Canada). p65-HA was from Tom Maniatis (Harvard, MA). The human Vigi1 promoter (450 bp) was cloned from Thp-1 genomic DNA into pGL-3 enhancer (Promega, Madison, WI).

**QRT-PCR.** Random-primed (Promega) cDNAs were generated by reverse transcription of total RNA samples with SuperScript II (Invitrogen). QRT-PCR analysis was performed with an iCycler iQ real-time PCR detection system (Bio-Rad, Hercules, CA) using 2 × QuantiTect SYBR Green PCR Kit (Qiagen). Standard curves used to determine the relative abundance of unknown samples were derived from serially diluted gel-purified PCR products or cloned plasmids. Data are expressed as fold changes of gene expression in LPS-treated samples compared with mock-treated samples. The expression of β-actin was used as a control. Primers were designed using Primer3 (Whitehead Institute/MIT Center for Genome Research, Cambridge, MA) or a public PrimerBank for PCR primers (49) (Table 1).

**ELISA.** BMDMs at day 8 ex vivo were plated and cultured in 96-well plates (5 × 10^4 cells/well) for 24 h prior to treatment with MALP2 (0.01, 0.1, 1, 10 nM), poly I:C (0.1, 1, 10, 100 μg/ml), LPS (0.1, 1, 10, 100 ng/ml), R-848 (0.01, 0.1, 1, 10 μM), CpG DNA

### Table 1. Primers

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward Primer (5′)</th>
<th>Reverse Primer (3′)</th>
<th>cDNA Product Size, bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cox2</td>
<td>GGCTGACAAGGAGCTGGGAAA</td>
<td>GCCGGTTCTCAGATTGAGG</td>
<td>318</td>
</tr>
<tr>
<td>IL-1β</td>
<td>CAGGAGGACAAGTACTACCTCA</td>
<td>AGGCACAGATATTTCGCG</td>
<td>219</td>
</tr>
<tr>
<td>IL-6</td>
<td>AACGAGTACAGCAGTTGAGA</td>
<td>GAGCATGGAAATGCGGTA</td>
<td>285</td>
</tr>
<tr>
<td>IP-10</td>
<td>AAGGCTGGTCCCTTTATTTTCTT</td>
<td>AAGCCTGTGGATTTTCAAG</td>
<td>200</td>
</tr>
<tr>
<td>Irf1</td>
<td>GGAAGGGAGGATAGGGGGACAG</td>
<td>ACTGACGTGGAGGGGCAAG</td>
<td>341</td>
</tr>
<tr>
<td>Irg1</td>
<td>CACTTAAAGGGACGGGACCA</td>
<td>GGAAGCAAGAAAACAAAACAA</td>
<td>337</td>
</tr>
<tr>
<td>M-CSF</td>
<td>ATGTGGACCATGAGGAGCCCTGG</td>
<td>GCGGAGGAGGAGCTCGTATG</td>
<td>303</td>
</tr>
<tr>
<td>MIP-1β</td>
<td>TGGGCTGGCAAGGGTCTTTC</td>
<td>GAGAGGAGCCTCCTGAGATG</td>
<td>282</td>
</tr>
<tr>
<td>PAI-1</td>
<td>AGTGTGGAAGCTGCTGCTT</td>
<td>TTTTGGAGCTGGTGTCTAC</td>
<td>306</td>
</tr>
<tr>
<td>RANTES</td>
<td>GCCCGAATCACTGAGCTCAGT</td>
<td>CTTTCCTCTGGTGGTGCG</td>
<td>218</td>
</tr>
<tr>
<td>TNFα</td>
<td>GGGCTGAGGAGGGGAGGCTC</td>
<td>GCGATGACGACGAGCTGGG</td>
<td>450</td>
</tr>
<tr>
<td>Ift1</td>
<td>TGCTGCGTCTAGTGTGTCTTT</td>
<td>GAGGAGATGGAAACCTGTGT</td>
<td>329</td>
</tr>
<tr>
<td>MIP</td>
<td>TGGTCTGGCTGCTGCTGCTG</td>
<td>GGGGTTTGACTTAAGT</td>
<td>233</td>
</tr>
<tr>
<td>Vig1-pending*</td>
<td>AACCCCGGGTGGTGGCATCTA</td>
<td>AACCCCGGGTGGTGGCATCTA</td>
<td>140</td>
</tr>
<tr>
<td>Ift1*</td>
<td>AGTGAGTGGACGCGGGAATCTT</td>
<td>TCTGAGTGGCTTTATGATGGCAA</td>
<td>188</td>
</tr>
<tr>
<td>Tyki</td>
<td>GGGGAGAATAGGAGTGGGG</td>
<td>GGCTGCTGGCATGGTGGG</td>
<td>354</td>
</tr>
<tr>
<td>β-Actin</td>
<td>TGGCGATGCGATGCGATGCGATGCGAAGAA</td>
<td>TGGCGATGCGATGCGATGCGAAGAA</td>
<td>675</td>
</tr>
</tbody>
</table>

*Sec Ref. 49.
(0.005, 0.05, 0.5, 5 μM), or infection with Sendai virus (0.1, 1, 10, 100 hemagglutination units/ml). Cell culture supernatants were removed at 0, 1, 2, 4, 6, 8, and 24 h and analyzed for the presence of TNFα, IL-6, IL-12/40, granulocyte-macrophage-colony stimulating factor (GM-CSF), macrophage-colony stimulating factor (M-CSF), RANTES, and IP-10 by ELISA according to the manufacturer’s recommendations (R & D Systems, Minneapolis, MN).

Transcription factor binding site identification. The sequences of genes represented on the microarrays, spanning the region from 1,000 bp upstream of the start site of transcription to 500 bp downstream of that site, were downloaded from http://siriusb.umdnj.edu:18080/EZRetrieve/index.jsp (55), and Repeat Masker (http://www.repeatmasker.org/) was used to mask out repeats. NF-κB and interferon-stimulated response element (ISRE) sites were defined by TRANSFAC 6.0 weight matrices (http://www.generegulation.com), and a similarity scoring method was used to find matching sequence sites. For each position along the sequence, a match score to the weight matrix minus the minimal possible score calculated for the inserted (or deleted) position, was larger than 85. A concentration of 10 ng LPS/ml was chosen vs. controls, A concentration of 10 ng LPS/ml was chosen to approximate the endotoxin concentration encountered when cells are treated with live E. coli bacteria at a density of ~1 bacterium per macrophage. We found 1,055 genes (7.9% of the 13,369 genes represented on the array) to be significantly induced or repressed (fold change > 1; P < 0.01; n > 4; Benjamini-Hochberg correction) in at least one of the four conditions tested. The Benjamini-Hochberg correction, when used at a significance level of P < 0.01, predicts that only 1% of the genes deemed significant by this test (<11 genes in this experiment) can be expected to be false positives. The LPS- and E. coli-induced gene expression profiles observed in WT macrophages closely resemble previously published LPS- and pathogen-induced gene programs in human macrophages and dendritic cells (3, 17, 29, 30, 39). Among the most highly responsive genes in our data set were cytokines, chemokines, and other proinflammatory mediators, including TNFα, IL-1β, IL-12/40, IL-6, Gro1, IP-10, MIP-1α, MIP-1β, RANTES, and COX-2.

Next we compared the E. coli vs. the LPS response by one-way ANOVA analysis. Surprisingly, only 13 genes (P < 0.01) responded differentially to the E. coli and the LPS treatment among the 1,055 genes determined to be responsive controls, or 4) MyD88-null BMDMs stimulated with living E. coli vs. controls. A concentration of 10 ng LPS/ml was chosen vs. controls, A concentration of 10 ng LPS/ml was chosen to approximate the endotoxin concentration encountered when cells are treated with live E. coli bacteria at a density of ~1 bacterium per macrophage. We found 1,055 genes (7.9% of the 13,369 genes represented on the array) to be significantly induced or repressed (fold change > 1; P < 0.01; n > 4; Benjamini-Hochberg correction) in at least one of the four conditions tested. The Benjamini-Hochberg correction, when used at a significance level of P < 0.01, predicts that only 1% of the genes deemed significant by this test (<11 genes in this experiment) can be expected to be false positives. The LPS- and E. coli-induced gene expression profiles observed in WT macrophages closely resemble previously published LPS- and pathogen-induced gene programs in human macrophages and dendritic cells (3, 17, 29, 30, 39). Among the most highly responsive genes in our data set were cytokines, chemokines, and other proinflammatory mediators, including TNFα, IL-1β, IL-12/40, IL-6, Gro1, IP-10, MIP-1α, MIP-1β, RANTES, and COX-2.

Next we compared the E. coli vs. the LPS response by one-way ANOVA analysis. Surprisingly, only 13 genes (P < 0.01) responded differentially to the E. coli and the LPS treatment among the 1,055 genes determined to be responsive controls, or 4) MyD88-null BMDMs stimulated with living E. coli vs. controls. A concentration of 10 ng LPS/ml was chosen vs. controls, A concentration of 10 ng LPS/ml was chosen to approximate the endotoxin concentration encountered when cells are treated with live E. coli bacteria at a density of ~1 bacterium per macrophage. We found 1,055 genes (7.9% of the 13,369 genes represented on the array) to be significantly induced or repressed (fold change > 1; P < 0.01; n > 4; Benjamini-Hochberg correction) in at least one of the four conditions tested. The Benjamini-Hochberg correction, when used at a significance level of P < 0.01, predicts that only 1% of the genes deemed significant by this test (<11 genes in this experiment) can be expected to be false positives. The LPS- and E. coli-induced gene expression profiles observed in WT macrophages closely resemble previously published LPS- and pathogen-induced gene programs in human macrophages and dendritic cells (3, 17, 29, 30, 39). Among the most highly responsive genes in our data set were cytokines, chemokines, and other proinflammatory mediators, including TNFα, IL-1β, IL-12/40, IL-6, Gro1, IP-10, MIP-1α, MIP-1β, RANTES, and COX-2.

Next we compared the E. coli vs. the LPS response by one-way ANOVA analysis. Surprisingly, only 13 genes (P < 0.01) responded differentially to the E. coli and the LPS treatment among the 1,055 genes determined to be responsive controls, or 4) MyD88-null BMDMs stimulated with living E. coli vs. controls. A concentration of 10 ng LPS/ml was chosen vs. controls, A concentration of 10 ng LPS/ml was chosen to approximate the endotoxin concentration encountered when cells are treated with live E. coli bacteria at a density of ~1 bacterium per macrophage. We found 1,055 genes (7.9% of the 13,369 genes represented on the array) to be significantly induced or repressed (fold change > 1; P < 0.01; n > 4; Benjamini-Hochberg correction) in at least one of the four conditions tested. The Benjamini-Hochberg correction, when used at a significance level of P < 0.01, predicts that only 1% of the genes deemed significant by this test (<11 genes in this experiment) can be expected to be false positives. The LPS- and E. coli-induced gene expression profiles observed in WT macrophages closely resemble previously published LPS- and pathogen-induced gene programs in human macrophages and dendritic cells (3, 17, 29, 30, 39). Among the most highly responsive genes in our data set were cytokines, chemokines, and other proinflammatory mediators, including TNFα, IL-1β, IL-12/40, IL-6, Gro1, IP-10, MIP-1α, MIP-1β, RANTES, and COX-2.
to either stimulus. This number nearly matches the number of genes whose expression would be predicted to differ by chance (~11 genes), and thus it appears that the E. coli and LPS stimulations produced virtually identical gene expression profiles. In Fig. 2D, the similarity in macrophage responses to LPS and E. coli is depicted using a self-organizing map (SOM) clustering algorithm (GEDI; Ref. 7) that graphically portrays the expression of the 1,055 responsive genes.

MyD88-dependent, MyD88-independent, and “MyD88-modulated” pathways mediate the LPS response. We next set out to group genes based on their requirement for MyD88. Genes can be either induced or repressed by LPS treatment, the response can be either MyD88 dependent or independent, and the MyD88 dependence can be directional: that is, the absence of MyD88 can either increase or decrease the LPS response. To populate these theoretical groups (illustrated in Fig. 2A), we started by determining which genes were induced or repressed following LPS stimulation in WT macrophages. For those genes whose expression changed, we next determined whether this change required MyD88 to be present. MyD88-dependent genes were initially defined as those whose absolute change in response to LPS was significantly reduced in the absence of MyD88 (P < 0.01, one-way ANOVA using Benjamini-Hochberg correction). The expression of a few genes that failed to reach this statistical measure of significance was noted to be consistently different in WT and MyD88-deficient cells. In

![Fig. 2. LPS signaling is mediated by MyD88-dependent, MyD88-independent, and MyD88-modulated pathways. The LPS response in wild-type (WT) and MyD88-deficient macrophages was classified into seven theoretical groups (A), defined by the WT response to LPS treatment (x-axis) and the MyD88 dependency of the response (y-axis). Fold changes in gene expression upon LPS treatment were compared with mock treatment, assessed by microarray analysis (B). The LPS response consisting of MyD88-dependent, MyD88-independent, and MyD88-modulated genes is dominated by MyD88-independent pathways. Percentages reflect the number of genes in the category shown divided by the total number of genes that responded to LPS (C). A graphical mosaic of LPS-responsive gene expression clusters produced by a self-organizing map algorithm (GEDI) illustrates the similarity of gene responses to LPS and E. coli (D).](image-url)
order not to exclude these genes, we added a second criterion by which a gene could qualify as MyD88 dependent. This criterion required that the difference in expression between MyD88-null and WT cells differed by >4-fold (average calculated for n > 4 replicates) upon LPS or E. coli treatment. In total, nine genes, including IL-12p40, were added to the MyD88-dependent gene list using this criterion (see P values for IL-12p40 in Table 2).

In the statistical analysis, we also noted a small group of genes that were significantly dependent on MyD88 but showed increased induction (or repression) in the absence of MyD88, rather than decreased induction, as might have been expected. Hence, if the absolute response to LPS was greater in the absence of MyD88, then the genes were classified as "MyD88 modulated" (Fig. 2, A and B). The remaining genes that were LPS responsive, but neither dependent nor modulated by MyD88 as defined above, were considered to be MyD88 independent.

About half of the genes were induced (507 genes of 1,055 or 48.1%) by LPS treatment and half were repressed. This 50% ratio was largely preserved when broken down into the various types of MyD88 responses as just defined (Fig 2A). However, Table 2. Representative genes of the categorized LPS-induced gene program in macrophages

<table>
<thead>
<tr>
<th>Gene</th>
<th>WT (n = 8)</th>
<th>MyD88 (n = 8)</th>
<th>Fold Change</th>
<th>P&lt;sub&gt;CHX&lt;/sub&gt; (n = 3)</th>
<th>Fold Change</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LPS-induced genes (fold upregulated)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interleukin-1β</td>
<td>29 (21–39)</td>
<td>44 (31–62)</td>
<td>0.43</td>
<td></td>
<td>22 (12–41)</td>
</tr>
<tr>
<td>Chemokine (C-X-C motif) ligand 10 (IP-10)</td>
<td>26 (17–40)</td>
<td>37 (18–74)</td>
<td>0.99</td>
<td></td>
<td>30 (20–44)</td>
</tr>
<tr>
<td>Colony stimulating factor 2</td>
<td>12 (9.3–16)</td>
<td>22 (13–38)</td>
<td>0.13</td>
<td></td>
<td>17 (15–20)</td>
</tr>
<tr>
<td>Serine (or cysteine) proteinase inhibitor, clade E, member 1 (Serpine1; PAI-1)</td>
<td>31 (15–62)</td>
<td>7.4 (5.1–11)</td>
<td>&lt;0.0001</td>
<td>2.8 (1.2–6.5)</td>
<td>0.047</td>
</tr>
<tr>
<td>Gene</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>LPS-repressed genes (fold downregulated)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interleukin-1β</td>
<td>73 (46–117)</td>
<td>9.3 (3.4–2.6)</td>
<td>&lt;0.0001</td>
<td>36 (16–77)</td>
<td>0.047</td>
</tr>
<tr>
<td>Prostaglandin-endoperoxide synthase 2 (Cox-2)</td>
<td>58 (19–183)</td>
<td>3.1 (1.5–6.2)</td>
<td>0.00032</td>
<td>14 (7.7–25)</td>
<td>0.20</td>
</tr>
<tr>
<td>Chemokine (C-X-C motif) ligand 1 (CXCL1; KC; Gro1)</td>
<td>54 (27–107)</td>
<td>23 (11–45)</td>
<td>0.0067</td>
<td>69 (49–97)</td>
<td>0.69</td>
</tr>
<tr>
<td>Interleukin-1α</td>
<td>45 (18–109)</td>
<td>8.9 (5.4–15)</td>
<td>&lt;0.0001</td>
<td>29 (21–41)</td>
<td>0.57</td>
</tr>
<tr>
<td>Tumor necrosis factor</td>
<td>35 (21–57)</td>
<td>12 (9.5–15)</td>
<td>&lt;0.0001</td>
<td>23 (12–43)</td>
<td>0.53</td>
</tr>
<tr>
<td>Serine (or cysteine) proteinase inhibitor, clade E, member 1 (Serpine1; PAI-1)</td>
<td>31 (15–62)</td>
<td>7.4 (5.1–11)</td>
<td>&lt;0.0001</td>
<td>2.8 (1.2–6.5)</td>
<td>0.047</td>
</tr>
<tr>
<td>Chemokine (C-X-C motif) ligand 2 (MIP-2)</td>
<td>30 (21–42)</td>
<td>5.4 (3.2–9.1)</td>
<td>&lt;0.0001</td>
<td>33 (20–54)</td>
<td>0.83</td>
</tr>
<tr>
<td>Chemokine (C-X-C motif) ligand 4 (MIP-1β)</td>
<td>24 (13–43)</td>
<td>7.4 (6.7–13)</td>
<td>&lt;0.0001</td>
<td>4.7 (2.9–7.5)</td>
<td>0.24</td>
</tr>
<tr>
<td>Interleukin-10 (IL-10)</td>
<td>23 (15–35)</td>
<td>5.4 (2.4–12)</td>
<td>0.00052</td>
<td>1.0 (0.8–1.2)</td>
<td>0.00023</td>
</tr>
<tr>
<td>Interleukin-12β (IL-12p40)</td>
<td>17 (8.5–34)</td>
<td>1.7 (0.2–13)</td>
<td>0.021</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colony stimulating factor 2</td>
<td>13 (6.3–25)</td>
<td>1.8 (1.3–2.3)</td>
<td>0.00044</td>
<td>2.8 (0.8–10)</td>
<td>0.39</td>
</tr>
<tr>
<td>Interleukin-6 (IL-6)</td>
<td>16 (7.3–32)</td>
<td>0.00016</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interleukin-1β</td>
<td>37 (31–62)</td>
<td>17 (9.9–25)</td>
<td>0.000050</td>
<td>63 (38–105)</td>
<td>0.19</td>
</tr>
<tr>
<td>Interleukin-6 (IL-6)</td>
<td>37 (20–44)</td>
<td>17 (11–25)</td>
<td>0.000050</td>
<td>63 (38–105)</td>
<td>0.19</td>
</tr>
<tr>
<td>Interleukin-11β</td>
<td>37 (20–44)</td>
<td>17 (11–25)</td>
<td>0.000050</td>
<td>63 (38–105)</td>
<td>0.19</td>
</tr>
<tr>
<td>Interleukin-12β (IL-12p40)</td>
<td>17 (8.5–34)</td>
<td>1.7 (0.2–13)</td>
<td>0.021</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colony stimulating factor 2</td>
<td>13 (6.3–25)</td>
<td>1.8 (1.3–2.3)</td>
<td>0.00044</td>
<td>2.8 (0.8–10)</td>
<td>0.39</td>
</tr>
</tbody>
</table>

Bone marrow-derived macrophages (BMDMs) were treated with 10 ng/ml of lipopolysaccharide (LPS) for 2 h, and their gene expression profiles were compared with that of mock-treated cells incubated for the same time using two-color oligonucleotide microarrays. Cells from four individual mice of each genotype were used. Each mouse served as its own mock control, and expression was assayed in duplicate with reversal of the dyes used for labeling control and stimulated samples (n = 8 microarrays per genotype). Cells from three wild-type (WT) mice were treated with 10 ng/ml of LPS and 10 μM cycloheximide for 2 h. The data are reported as fold changes upon LPS treatment, compared with mock treatment. Average fold changes are followed by a 95% confidence interval within parenthesis. Probability values for the effect of MyD88 deletion (P<sub>CHX</sub>) and the effect of cyclohexamide inhibition of the LPS response in WT macrophages (P<sub>CHX</sub>) were calculated by r-test.

Physiol Genomics • VOL 19 • www.physiolgenomics.org.
those genes whose expression was induced had larger percentage changes in expression than the genes that were repressed (note the distribution along the x-axis in Fig. 2B).

**MyD88-independent gene expression dominates the LPS response.** Although recent studies (22, 24, 38) had prepared us for the possibility that a few genes would not require MyD88 for their activation by LPS stimulation, we expected the number to be quite small and anticipated that those might be worth analyzing by individual experimental interrogation. In contrast to this prediction, 788 (74.7%) of the 1,055 genes that met our criteria as LPS responsive were MyD88 independent. Only 227 (21.5%) genes were MyD88 dependent (Fig. 2C). The number of MyD88-modulated genes was small (40 genes or 3.8%). A striking feature of the data is the predominance of genes induced or repressed to the same extent by LPS in WT and MyD88-deficient cells. As our criteria for classification of genes as MyD88 dependent were quite stringent, we thought it possible that they might have led to the overestimation of the genes classified as MyD88 independent. We therefore examined the levels of differential gene expression in the subset of genes classified as MyD88 independent. This analysis demonstrated that more than 82% of these genes had an average LPS-stimulated expression level in the WT macrophages that was <30% different from the MyD88-deficient macrophages. With current microarray methodology, expression differences of this magnitude typically represent variability in the assay measurement rather than significant differences in gene expression. Thus our finding of the predominance of MyD88-independent gene expression in response to LPS stimulation is unlikely to be a consequence of our choice of criteria in defining MyD88 dependence. The predominance of MyD88-independent gene expression is readily visualized in the GEDI mosaic graph (Fig. 2D), where the majority of colorized clusters persists in the MyD88-null cells compared with the WT macrophages. Thus LPS induces a vast gene expression program that is independent of MyD88 activity, although many of the best characterized LPS responses do, in fact, require MyD88.

**MyD88-dependent pathways regulate many classic markers of inflammation.** The expression of many well-characterized markers of inflammation, including IL-1β, Cox-2, TNFα, IL-10, IL-12, and IL-6, required MyD88 for optimal expression (Table 2). We measured the MyD88-dependent expression of TNFα, MIP-1β, IL-1β, PAI-1, Cox-2, and IL-10 using QRT-PCR (Fig. 3A) to verify the microarray results. Simultaneous measurement of the housekeeping gene, β-actin, indicated no change in its expression in the comparison of WT to MyD88-deficient macrophages (Fig. 3B). The protein expression of many of the MyD88-dependent inducible genes was also measured by ELISA, to further validate the RNA expression results. As seen in Fig. 3C, TNFα, IL-6, IL-12p40, and GM-CSF production was severely attenuated in macrophages taken from MyD88-deficient mice. Thus the microarray data for MyD88-dependent expression of several of the most important markers was verified at the protein level. This finding, in association with the microarray data, serves to establish the role of MyD88 in regulating many markers of inflammation.

![Fig. 3. QRT-PCR and protein expression analysis of MyD88-dependent gene expression.](image-url)
macrophage inflammatory genes was confirmed using independent measurements of both mRNA and protein production. 

*MyD88-independent pathways regulate known inflammatory responses as well as many unknown genes.* Several of the most highly induced genes were found to be MyD88 independent (Table 2). Some of these genes have previously been shown to participate in the immune response, such as Irf1, IP-10, RANTES, and M-CSF, and their expression was confirmed by QRT-PCR (Fig. 4A). However, the functions of many of the MyD88-independent genes are unknown. In the very large group of genes determined to be MyD88 independent, only IP-10, Irg1, IFN-β, and Ifit1 have previously been reported to respond to LPS stimulation via MyD88-independent signaling pathways (24). Using QRT-PCR analysis, we verified the MyD88-independent expression of several genes not previously known to fall into this category, including Irf1, MARCKS-like protein (Mlp), and M-CSF (Fig. 4A). ELISA analysis of RANTES and M-CSF established that protein expression also mirrored the mRNA data for MyD88-independent genes (Fig. 4B).

BMDMs were cultured in cytokine-rich medium for several days prior to experimentation. The possibility existed that the large number of genes expressed independently of MyD88 activity that we observed reflected the ex vivo maturation conditions used in the culturing of the BMDMs. Thus we measured the LPS-induced activation of two MyD88-dependent genes (TNFα and IL-1β) and two MyD88-independent genes (RANTES and Irf1) in thioglycolate-elicited peritoneal-exudate macrophages by QRT-PCR to assess their dependence on the macrophage preparative conditions (Fig. 3D and 4C). Although the magnitudes of the MyD88-dependent gene responses were at least 10-fold higher in the peritoneal macrophages than in the BMDMs, the MyD88-dependent and -independent classifications did not differ between these macrophage preparations.

*MyD88-modulated genes reveal a novel regulatory function of MyD88.* A third group of genes identified in these experiments were those whose expression was induced by LPS to higher levels or repressed to lower levels in macrophages.
lacking MyD88 activity. These we termed “MyD88 modula-
ted.” The modulation of viral hemorrhagic septicemia virus (VHSV) induced gene 1 (Vig1-pending, also known as Viperin and cig5), interferon-induced gene with tetratricopeptide repeats 3 (Ift3), and thymidylate kinase (Tyki) was confirmed using QRT-PCR (Fig. 5A). The QRT-PCR data suggests there may be more MyD88-modulated genes than can be classified using microarray data. Both Ift1 and Ifr1 were classified as MyD88 independent because their expression levels did not differ significantly, using our chosen cutoff value (P < 0.01).

With QRT-PCR methodology, however, these would have been categorized as MyD88 modulated (Fig. 4A and Table 2). The existence of MyD88-modulated genes was further established by measuring LPS induction in WT and MyD88-deficient mouse embryonic fibroblasts (MEFs) by QRT-PCR (Fig. 5B). The LPS- and poly I:C-induced response was greater in MyD88-deficient MEFs compared with MyD88-containing MEFs. Both IL-6 and RANTES protein expression measured by ELISA were induced by LPS in WT MEFs (data not shown).

Although the MyD88-modulated gene group comprised only a small percentage (3.8%) of the LPS-responsive genes on the arrays, they provided an insight into a potentially novel MyD88 function. This function can be viewed as constraining the responsiveness of genes (either induction or repression) to endotoxin stimulation. To investigate whether MyD88 modulation also occurs downstream of TLR-3, a Toll receptor whose signaling appears to be entirely dependent on the expression of TRIF, we treated WT and MyD88-deficient MEFs with the double-stranded RNA mimetic poly I:C for 2 h and assayed the induction of MyD88-modulated genes by QRT-PCR (Fig. 5B). The expression of Vig1-pending, Ift3, and Tyki was increased in the absence of MyD88 after poly I:C stimulation, suggesting that MyD88 may also influence the expression of genes induced by the TLR-3 pathway.

MyD88-independent gene expression does not depend on new protein synthesis. Interferon-responsive genes (IRGs) are components of an anti-viral signaling cascade shared by TLR-3 and TLR-4 and are regulated, in part, by the transcription activator, interferon regulatory factor (IRF)-3 (4). The type I interferon IFN-β is an example of one such gene, whose expression can activate a second wave of gene expression via the type I interferon receptor and the JAK/STAT pathways (4, 45). As only one (myxovirus resistance protein 1; IFN-β) of 19 LPS-responsive genes annotated as viral response genes or IRGs was MyD88 dependent, we wondered whether the induction of many of the MyD88-independent genes could be explained by secondary transcriptional events that were dependent on the generation of protein produced in the initial wave of gene induction. To test this possibility, WT macrophages were treated with LPS in the presence of 10 μM of the protein synthesis inhibitor cycloheximide (26). Analysis of this issue was limited to genes whose average expression changed at least fourfold or more in response to LPS, as determined in three or more independent microarray experiments. Genes were deemed cycloheximide sensitive if the induction by LPS alone or by LPS plus cycloheximide was significantly different (t-test; P < 0.05). Furthermore, gene repression, should it exist for a given gene or set of genes, is often reduced or eliminated by cycloheximide treatment as transcripts become protected from degradation, resulting in enhanced stability of mRNAs (26). We found that the majority of those genes whose expression was repressed by endotoxin were not repressed in the presence of cycloheximide (15 of 18 genes or 83%; Table 2). In contrast, the majority of those genes that were unregulated after LPS stimulation were not sensitive to cycloheximide (65 of 97 genes or 67%; Table 2). MyD88-independent genes still constituted the majority of LPS-responsive genes in the cycloheximide-treated cells (41 of 65 genes or 63%), establishing that MyD88-independent signaling does not simply reflect widespread secondary transcriptional events. These data confirm previous studies by others showing that IP-10 and Ifr1 are primary response genes (4, 12) and classifies the expression of several other genes as part of a primary or secondary response pathways (Table 2).

Vig1-pending and other genes not requiring MyD88 for maximal induction are regulated by concordant NF-κB and IRF-3 activation. We were particularly interested in MyD88-independent antiviral response genes such as RANTES, IP-10, and Ift1, which can be activated without new protein synthesis and are known to be induced by coordinate IRF-3 and NF-κB activation. This suggested that the presence of IRF-3 and NF-κB transcription factor binding sites might represent a signaling module activating at least a subset of the MyD88-

![Fig. 5. QRT-PCR expression analysis of MyD88-modulated genes. Total RNA was isolated from macrophages 2 h after stimulation with 10 ng/ml LPS or mock treatment. The fold change in gene expression was determined by comparison to the mock-treated sample using QRT-PCR (A). Total RNA was isolated from mouse embryonic fibroblasts (MEFs) 2 h after stimulation with 100 ng/ml LPS (B) and 100 μg/ml poly I:C (C), and the fold change in gene expression (compared to a mock-treated sample) was quantified by QRT-PCR.](on March 28, 2011 physiolgenomics.physiology.org Downloaded from)
independent genes. Hence, we examined the promoter regions of all of the genes arrayed on our slides for both NF-κB and IRF transcription factor binding sites. The known IRF-3/ NF-κB response genes (RANTES, IFNβ, IP-10, and Ifit1) contained both ISRE and NF-κB sites within 300 bp of the transcription start site and no greater than 100 bp apart from each other. In addition, Irg1, Vig1-pending, and Ifit3, which respond robustly to LPS, share these same tandem site characteristics, suggesting that they are also regulated by concordant IRF-3 and NF-κB activation (Fig. 6A).

To explore the concordant IRF-3 and NF-κB activation further, we examined the inducibility of the Vig1-pending promoter, a gene strongly induced by LPS, in HEK293 cells expressing TLR-3. As seen in Fig. 6B, two strong activators of NF-κB, IL-1β and TNFα, both failed to induce Vig1-pending reporter gene activity. In contrast, stimulation of cells with poly I:C or infection with Sendai virus, which activate both NF-κB and IRF-3, strongly induced reporter gene activity, suggesting that activation of NF-κB alone is insufficient to induce Vig1-pending promoter activation. Recently the TIR domain-containing adapter molecules TRIF and TRAM have been shown to regulate MyD88-independent gene expression via IRF-3. Overexpression of either of these two adapters or MyD88 results in the activation of their downstream signal transduction pathways (8). We examined the ability of either MyD88 or TRIF to activate the Vig1-pending reporter gene (Fig. 6C) in transfected HEK293 cells. Overexpression of MyD88 did not induce Vig1 promoter activity, whereas overexpression of TRIF induced a vigorous Vig1 promoter response. Vig1 promoter activity was also induced by the constitutively active IRF3–5D mutant (Fig. 6D), but not by the p65 subunit of NF-κB, again suggesting that activation of NF-κB alone is insufficient to drive this promoter. This is in agreement with the inability of either IL-1 or TNFα to induce Vig1, as

![Fig. 6. A: Vig1-pending is activated by NF-κB and IRF-3. Schematic representation of NF-κB and ISRE transcription factor binding sites in LPS-induced genes. B: TLR-3-expressing HEK293 cells were transfected with luciferase reporter genes under the control of Vig1-pending and NF-κB promoters and activated by IL-1β, TNFα, Sendai virus, and poly I:C. C: Vig1-pending and NF-κB promoter activation when cotransfected with MyD88 or TRIF. D: Vig1-pending and NF-κB promoter activation when cotransfected with constitutively active IRF-3 or p65.](http://physiolgenomics.physiology.org/issue)
these cytokines activate NF-κB but will not activate reporter gene expression from an ISRE. Furthermore, the induction of Vig1 by TRIF was inhibited by coexpression of dominant-negative forms of both NF-κB and IRF signaling (the 1κB super-repressor or IRF-3 dominant negative) (data not shown). Taken together, these observations confirm that the MyD88-independent pathway regulator, TRIF, induces Vig1 and that expression of Vig1 requires activation of both NF-κB and IRF-3. Although several MyD88-independent genes contained NF-κB and ISRE binding sites, and are likely critical early mediators of MyD88-independent signaling events, it is clear that additional signaling pathways remain to be identified that would account for the expression activity of the large number of MyD88-independent genes that apparently lack proximal ISRE binding sites.

DISCUSSION

The present study illustrates the use of expression profiling to compare and classify the LPS response in cells that do and do not express critical mediators of that response. This study has some inherent limitations that must be recognized. To conduct these experiments, it was necessary to select a single time point to perform the comparison. As previous work has indicated that LPS responses can be delayed rather than lost in mice lacking key mediators in the TLR pathway (23), our classifications of genes as MyD88 dependent or independent are necessarily limited to the time course we have chosen. To fully understand the complex signaling network induced by LPS over time, additional time course experiments will be required. In addition, our understanding of the transcriptional regulation of this gene network is quite rudimentary, making it unwise to generalize from the limited Vig1-pending transcriptional analysis undertaken here.

In view of the toxicity of gram-negative bacteria, it is not surprising that LPS activates such a rich gene expression program (~1,000 genes or almost 8% of the genes probed in this experiment), all of which are likely induced via TLR-4. The data presented here show that LPS and E. coli induce almost identical gene expression profiles, suggesting that the contribution of TLRs other than TLR-4 to the E. coli response is either minor or overlapping with the TLR-4 response. The data are the latest confirmation of a long-held (but nevertheless controversial) dogma, i.e., that the LPS portion of the outer leaflet of gram-negative bacteria is the predominant activator of the innate immune system. In this respect, the influence of endotoxin appears to be far greater than other cell wall constituents, including immunologically important molecules such as lipopeptide, peptidoglycan, and flagellin.

MyD88-deficient mice do not need to be housed in a pathogen-free environment to survive and have been shown to mount a host defense response to polymicrobial peritonitis (51) and local Staphylococcus aureus infection, despite blunted cytokine responses (41). This suggests that MyD88-independent signaling pathways play important roles in the control of endogenous host microflora as well as in the defense against some virulent pathogens. However, MyD88-deficient mice are more vulnerable to certain infections, such as systemic challenge with S. aureus (46), Listeria monocytogenes (6), and Pseudomonas aeruginosa (41), indicating that MyD88 plays a vital role in normal host defense against important pathogens. The genes downstream of the NF-κB pathway are clearly critical to this host defense response and were clearly dysregulated in the absence of MyD88.

NF-κB, JNK, and p38 MAPK are essential for the induction of TNFα and are activated downstream of MyD88 (40, 42). In MyD88-deficient cells, NF-κB, JNK, and p38 MAPK all exhibited delayed activation. The lack of LPS-inducible TNFα protein production in MyD88-null cells may be due to this delayed activation, although it is difficult to understand how the modestly altered kinetics of these responses might translate into such a profound defect in protein production, especially when so many other LPS responses are entirely intact. Alternatively, MyD88 might regulate an unknown key factor essential for TNFα transcription, mRNA stabilization, translation, or secretion. It should also be noted that not all reports (47) have shown the same failure of MyD88-deficient macrophages to secrete cytokines in response to LPS as presented here and elsewhere (23). Our data also suggest that results obtained in macrophages from different sources by different experimental protocols may not always be directly comparable. The use of thioglycolate to elicit the macrophage exudate in the peritoneum may result in cells that are primed to be more responsive to subsequent inflammatory stimuli. Such sensitization has been observed, for instance, for IL-6 production in macrophages when primed with either low doses of LPS (13) or M-CSF (43).

To avoid detrimental uncontrolled inflammation, the innate immune response must constantly strike a balance between activation and inhibition. Following LPS stimulation, macrophages enter a transient state where a second LPS stimulus is blunted or absent. This so-called “endotoxin tolerance” suggests that inhibitory pathways may become activated that downregulate the LPS response. The “MyD88 modulation” phenomena described herein may reflect a requirement for MyD88 in this attenuation response. MyD88 could directly inhibit LPS activation pathways or alternatively could be required for the induction of an inhibitory molecule. Recently a number of negative regulators of LPS signaling have been described that could modulate expression by either of these mechanisms. These proposed negative regulators, including a short splice variant of MyD88 (MyD88s) (20), Irak-M (25), SOCS-1 (28), PI3K (11), and the TIR domain containing receptors T1-ST2 (44) or SIGGIR (48). These proteins represent attractive candidates responsible for the MyD88 modulation observed in this study.

Our study suggests that the majority (3/4) of the host response to LPS is regulated independently of MyD88 and that this response involves a diversity of host activities in addition to the well-characterized type I interferon response. LPS signaling appears to uniquely utilize all four of the known TIR domain-containing adapter molecules [MyD88, Mal (also known as TIRAP) (9, 16), TRIF (also known as TICAM-1) (33, 54), and TRAM (also known as TIRP/TICAM-2) (2, 10, 34, 53)] to activate its full transcriptional response. Published reports indicate that TRAM may be the “master adapter” for LPS responses (53). TRAM contains an N-myristoylation site that targets this adapter to TLR-4 in resting cells (D. C. Rowe, K. A. Fitzgerald, D. T. Golenbock, unpublished observations). Both targeted deletion and small interfering RNA (siRNA) silencing experiments have demonstrated a nearly absolute requirement for TRAM in the TLR-4 pathway (10, 53).
In contrast to MyD88 and Mal, which are thought to function as a heterodimer to regulate MyD88-dependent pathways, TRIF seems to bridge both MyD88-independent and MyD88-dependent pathways, TRIF-deficient (52) and TRIF-mutant mice (15) fail to induce IFNβ, IP-10, Irf1, RANTES, TNFα, IL-6, and IL-12p40. TRIF has been shown to interact with TRAF6, a component of the MyD88-dependent pathway that activates NF-κB and controls the expression of genes such as TNFα and IL-6 (21, 37). A recent study from Holzmann and coworkers (50) examining the gene expression profile following LPS stimulation in dendritic cells taken from mice carrying a loss of function mutation in TRIF found that ~50% of the LPS response was dependent on TRIF. Genes appearing to be TRIF-dependent are classified as both MyD88-independent and MyD88-dependent genes in our study, consistent with the ability of TRIF to bridge both MyD88-independent and MyD88-dependent pathways.

A genome-wide approach will be critical to defining the individual contribution, and possible redundancy, of the different adapter molecules and pathways to the LPS-induced response in immune cells. Once established, the differences in adapter molecule utilization can be exploited for the rationale design of anti-inflammatory therapies that inhibit certain subtypes of deleterious inflammatory responses while leaving host defense mechanisms intact. The data we report here provide an initial roadmap to defining the adapter specificity of the host innate immune response.

ACKNOWLEDGMENTS

We thank Dr. Gabriel S. Eichler for use of the Java version of the GEDI software v. 2.0.5.

GRANTS

This work was supported by the National Institutes of Health Grants DK-50305, HL-45098, and HL-66678 (to M. W. Freeman); and Grants GM-54060 and RR-14466 (to D. T. Golenbock). H. Björkbacka and K. A. Fitzgerald are supported by the Swedish Research Council and the Wellcome Trust, respectively.

REFERENCES


