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Gene Expression Profile of THP-1 Cells Infected by *R. prowazekii* Suggests Host Response Signature Genes

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Abstract: *Rickettsia prowazekii,* a selective agent of category B, is the causative microorganism of epidemic typhus. The genome-wide profile of host response to *R. prowazekii* infection was studied in THP-1 cells by a human cDNA microarray. Approximately 131 (1.71%) and 11 (0.14%) genes out of 7,680 genes assessed were up-regulated or down-regulated upon infection with virulent *R. prowazekii* strain Breinl. These genes induced by *R. prowazekii* were diverse in function. Six genes [ENG (endoglin or CD105, cell surface glycoprotein), GADD45A, TNFAIP3, IGFBP3, POU3F4 (transcription), ELK3] were identified as commonly induced genes as their over expressions were observed throughout the entire time course studied. There were twenty-two genes [such as GADD45A, POU3F4, ENG, PPP1R14B (an enzyme), ELK3, CXCL1, IL1B, NFKB1A, etc.] that exhibited the high level of induction at more than one time point. Collectively, these discoveries may provide novel insights into mechanisms of rickettsial pathogenesis and might reveal potential therapeutic targets against rickettsial infection.

INTRODUCTION

*Rickettsia*e are obligate intracellular Gram-negative bacteria. Rickettsial infections can cause acute fever, headache, skin rash, eschars, and in severe cases failure of the cardiovascular system. *R. prowazekii*, the causative agent of epidemic typhus, caused millions of deaths in both World Wars [1] and has reemerged worldwide recently [2]. It has been listed as select agent by the Centers for Disease Control and Prevention in the United States.

The host-rickettsia interaction is likely to involve a complex interplay between host defense and bacterial evasion as seen in other bacterial infections [3]. Based on the genome sequence of *R. prowazekii* Madrid E [4], the comparative genome-wide analysis of *R. prowazekii* virulent and avirulent strains (Breinl vs. Madrid E) revealed potential virulence genes that may provide useful information on the pathogenesis of *Rickettsia* [5]. Adherence to the membrane of target cell is a first key step in the establishment of a rickettsial infection. The increased expressions of the receptors E-selectin, intercellular adhesion molecules 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) have been reported in human endothelial cells post rickettsial infection [6]. It is believed that after adherence, rickettsiae enter into host cells and then rapidly escape from phagocytic vacuoles before phagolysosomal fusion [7]. Within the cytoplasm, rickettsiae induce rearrangement of host actin filaments in order to facilitate their intracellular spread and host cell locomotion [8]. A recent study of *R. conorii* has shown that the parasite surface protein, RickA, can recruit and activate Arp2/3, which then induces actin polymerization of host cells [8]. However, there is not much known about the sequence of events and molecules involved in rickettsial infection. The expression profile of host response to *R. prowazekii* infection has not been well investigated. With the availabilities of the human genome sequence and cDNA microarray technology, study of expression patterns on a global level has now become possible.

The aim of this study was to explore the host response to *R. prowazekii* infection by determining the global transcriptional response of a human monocytic cell line (THP-1 cells) upon infection. Human THP-1 cells in culture were harvested at various times post infection with *R. prowazekii*. The transcriptional expressions of THP-1 cells were analyzed on microchips containing 7,680 human cDNAs. From data mining and confirmation by quantitative RT-PCR, a characteristic pattern of responsive genes was identified. These results may yield insights into the mechanism of the human defense response to *R. prowazekii* infection.

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MATERIALS AND METHODOLOGY

Host Cell Culture and Exposure to *R. prowazekii* Virulent Strain Breinl

THP-1 cells originally derived from an acute monocytic leukemia patient were purchased from ATCC. The cells were cultured in RPMI 1640 with 10% FBS for several days until the cell density reached 1-2x10^7 cells in 162 cm^2 flask at 37°C, 5% CO_2. *R. prowazekii* Breinl were inoculated into THP-1 cells with multiplicities of infection around 25:1. The cultures were rocked at room temperature for one hour after which medium was removed and fresh medium was added, then the culture was returned to 37°C, 5% CO_2.

Total RNA Isolation

The cultures were harvested at 1, 4, 8, 18, and 24 hours (T1, T4, T8, T24) post-infection. Total RNA was extracted immediately using TRIzol (Invitrogen, Carlsbad, CA) following the protocol provided by the manufacturer. RNA was further purified using the RNeasy Mini Kit according to the manufacturer's instructions (Qiagen, Valencia, CA) with an additional step of on-column DNase Digestion using RNase-free DNase (Qiagen, Valencia, CA) to completely remove DNA. The yield, integrity, and purity of total RNA were determined by agarose gel electrophoresis and the ratio of O.D. 260/280.

Probe Labeling, Microarray Hybridization, and Image Analysis

The microarray slides used for this study contained 7,680 human cDNA clones (National Cancer Institute ROSP 8K Human Array) purchased from Research Genetics (Huntsville, AL). The cDNAs were spotted onto poly-L-lysine-coated slides by a computerized OMNI Grid Arrayer (GeneMachines, San Carlos, CA). THP-1 cells at T1-T24 post-infection and un-infected cells at corresponding time points were collected. Equal amount of RNAs (20-40 μg) from infected and uninfected cells were reverse transcribed and labeled with fluorescent Cy3 or Cy5 analogs of dCTP, respectively (Amersham, Piscataway, NJ). The labeled cDNAs were purified, concentrated, and hybridized onto microarray slides.

The microarrays were scanned at a 10-μm resolution on a GenePix 4000A scanner (Axon Instruments, Inc., Foster City) to obtain maximal signal intensities with < 1 % probe saturation. The Cy5- and the Cy3-labeled cDNA samples

Table 1. Primers and Probes Used in Real-Time PCR

Target Sequence (5'-3')
GADD45A
Forward……..5' TGT GAG TGA GTG CAG AAA GCA G 3'
Reverse…….5' CCA CCT TAT CCA TCC TTT CGG T 3'
Probe……..5' TCT GCT CTC CAG CCG AGA ATT CCT CCA A 3'
PPP1R14B
Forward…… ……5' GGA AGG TCA CCC TCA AGT ATG A 3'
Reverse…….. 5' TCA TCC ACG TCA ATC TCC AGT T 3'
Probe……5' TGG GAT CTC CTC TTC CTG GCA GTC GTA 3'
ENG
Forward……..5' CAA CAT GGA CAG CCT CTC TTT C 3'
Reverse……5' TGT CTA ACT GGA GCA GGA ACT C 3'
Probe……..5' CTC TAC CTC AGC CCA CAC TTC CTC CA 3'
NFKB1A
Forward……5' AGA GAG TGA GGA TGA GGA GAG 3'
Reverse……...5' ACA CAG TCA TCA TAG GGC AG 3'
Probe……5' TGT GAA CTC CGT GAA CTC TGA CTC TGT 3'
ILB1
Forward……5' ATT CTC TTC AGC CAA TCT TCA TT 3'
Reverse……..…5' GCC ATC AGC TTC AAA GAA CA 3'
Probe……5' TCA TCC TCA TTG CCA CTG TAA TAA GCC A 3'
CXCL1
Forward……5' CAA AGT GTG AAC GTG AAG TCC C 3'
Reverse……5' TGT TCA GCA TCT TTT CGA TGA TTT 3'
Probe……5' CTG CGC CCA AAC CGA AGT CAT AGC C 3'
TNFAIP3
Forward……5' ACA GAC ACA CGC AAC TTT AA 3'
Reverse……5' TTT GAT AAG ATT GTC CCA TTC ATC 3'
Probe……...5' CCG CTG GCA ACT GGA GTC TCT 3'

were scanned at 635 nm and 532 nm, respectively. The resulting TIFF images were analyzed by Gene Pix Pro 4.0 software (Axon Instruments, Inc., Foster City). Microarray data were stored in the NIH microarray database. The ratios of intensities of the infected to control samples for all targets were determined after background subtraction. Raw intensity profiles were analyzed using the mAdb tools (National Center for Biotechnology Information, NIH). The data were further analyzed by using TreeView and Cluster [9]. Ingenuity Pathway Analysis (IPA) [10-11] was applied to the data as an additional method to further evaluate the functional significance of genes. IPA uses a curated database to construct different regulatory networks of imported genes. Each gene identified by IPA was mapped to its corresponding gene object in the Ingenuity Pathway Knowledge Base. This program uses its knowledge base to identify interactions between focus genes and other related genes in order to create networks. IPA produces a statistical score for each network according to the fit of the network to the set of focus genes. The score is the negative log of p and indicates the likelihood of the focus genes in the network due to random chance. A core of 3 means that there is a 1/1000 chance that the focus genes are in a network due to random chance. Therefore, the scores of 3 or higher have a 99.9% confidence of not being generated by random chance alone. This score was used as a cut-off point for identifying gene networks that are significantly regulated by *R. prowazekii* infection.

Reverse Transcription and Real Time RT-PCR

RT was carried out using MessageSensor™ RT Kit (Ambion), which was specifically designed for elevated sensitivity in real time PCR. Real time PCR primer pairs and probes for selected genes were designed using TaqMan Design 2.0 software (ABI, Weiterstadt, Germany) and are shown in Table **1**. The sequences of exon and intron of those genes were searched and either primer or probe covered exon/exon junction to avoid the amplification of genomic DNA. The procedures for real time PCR were performed as described previously [5]. The probes were labeled with 6-carboxyfluorescein (FAM) and the new generation quencher (BHQ-1) at the 5' and 3' ends, respectively, by Biosearch Technologies (Novato, CA). PCR cycling was conducted using a SmartCycler (Cepheid, Sunnyvale, CA) in a total volume of 25 µl containing 1x TaqMan Universal PCR Master Mix (Applied Biosystems), 0.2 µM of each primer, 0.1 µM probe, and 1 µl of diluted cDNA template. The probe and primers for 18 S rRNA gene purchased from Biosource International were included as an endogenous reference. The comparative C_T (threshold cycles) method was applied using arithmetic formulas ($2^{-\Delta\Delta C_T}$) (ABI system, AB, Piscataway, NJ). PCR reactions with no-cDNA templates were included as negative controls. Real time RT-PCR assays were performed in triplicate for each sample at every time point, and a mean value and standard deviation were calculated for the relative RNA expression levels.

RESULTS

Hierarchical Clustering of Signature Genes and their Functional Categories

To characterize the host gene response to *R. prowazekii*, a human cDNA microarray containing 7,680 genes was used. THP-1 cells infected with *R. prowazekii* were harvested at T1-T24. Total RNA was extracted for cDNA synthesis. To exclude the possibility of cross hybridization of bacteria cDNA to human microarray slides, poly dT was used as primer for Cy3 or Cy5 incorporation reaction instead of random primers in all the labeling procedures since rickettsial mRNA lacks poly A at 3' end. The differences in signal intensities of co-hybridization experiments were compared for each spot between infected and uninfected control cells.

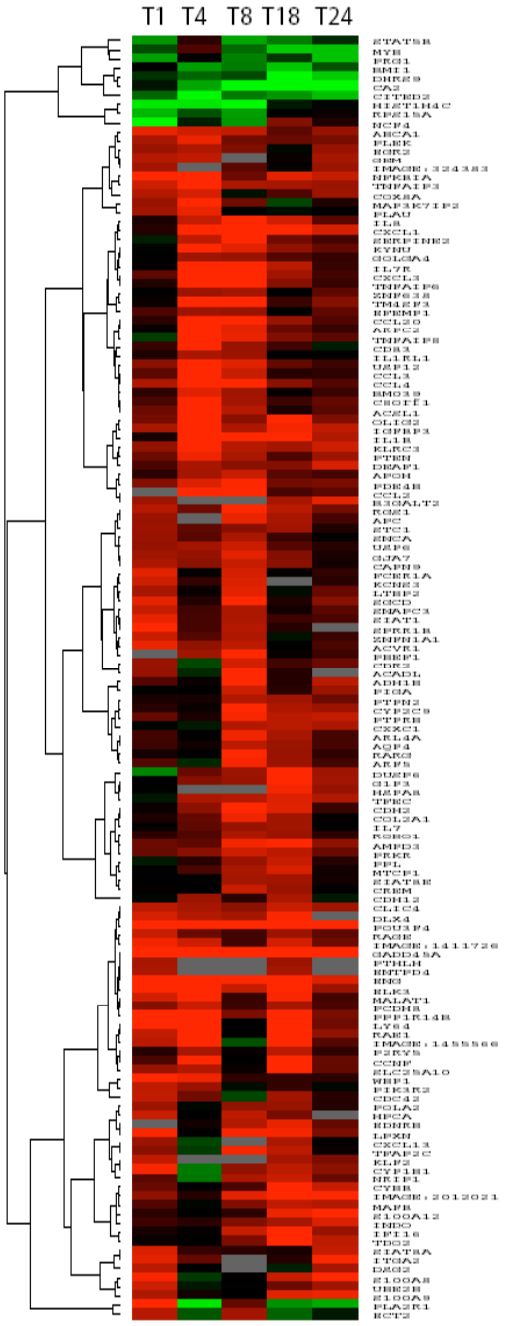

Fig. (1). H. cluster image of 142 genes with fluorescence ratios of ≥ 1.5 or ≤ 0.667 at any two time points compared to uninfected control. Lane 1 to 5 indicates the time course at T1-T24 after infection. The genes are ordered by similarity in their expression patterns across the time course. Color indicates relative expression intensity (red, increased; green, decreased; and grey, missing data).

Table 2. 142 Genes Up-Regulated by ≥ 1.5 Fold or Down-Regulated by ≤ 0.667 at More than One Time Points Post-Infection

Induced (131)			**Repressed (11)**
Cytokines (4)	Receptor (11)	Apoptosis/growth arrest (3)	PRG1
IL1B	IL7R	PRKR	CA2
IL1RL1	P2RY5	PTEN	BMI1
G1P3	EDNRB	GADD45 α	STAT5B
PBEF1	PTPN2		NCF4
	PTPRB	Proliferation (3)	PLA2R1
Chemokines (8)	RARG	CCNF	RPS15A
IL8	ACVR1	MTCP1	HIST1H4C
MIP1B/CCL4L	SERPINE2	IGFBP3	MYB
CXCL1/GRO1	FCER1A		CITED2
CXCL3/GRO3/MIP2B	LY64	Enzyme (21)	DHRS9
CXCL13/BLC	IL27RA	KYNU	
CCL2/MCP1		INDO	
CCL3/MIP1A	Signalling (14)	AMPD3	
CCL20	PTHLH	USP12	
	MAP3K71P2	USP32	
Adhesion (10)	PIK3R2	UBE2B	
ENG	RGS1	SIAT8A	
LPXN	PDE4B	SIAT8E	
APC	ARF5	SIAT1	
TNFAIP6	TM4SF3	ENTPD4	
ITGA2/CD49B	LTBP2	ACADL	
PCDH8	CDC42	PPP1R14B	
CDH2	ARL4A	COX8A	
CAPN9	GEM	POLA2	
ROBO1	HSPA8	ADH1B	
CDH12	DUSP6	ACSL1	
	ECT2	CYP2C9	
Cytoskeletin/motility (7)		CYP1B1	
ARPC2	Transcription (14)	TDO2	
SGCD	NFKB1A	ENTPD4	
SPRR1B	NRIP1	B3GALT2	
C8orf1	TFAP2C		
COL2A1	OLIG2	Miscellaneous (20)	
PPL	CREM	CD83	
HPCA	Ikaros	KLRC2	
	KLF2	CYBB	

(Table 2). Contd…..

Induced (131)			Repressed (11)
Transporter (6)	POU3F4	DLX4	
AQP4	IFI16	EGR2	
KCNS3	ELK3	MAFB	
SLC25A10	TFEC	CDR2	
CLIC4	CXXC1	C6orf142	
ABCA1	ZNF638	DEAF1	
APOH	SNAPC3	RAE1	
		BM039	
Calcium ion binding (7)	Anti-apoptotic (3)	WBP1	
EFEMP1	TNFAIP3	MALAT1	
STC1	TNFAIP8	PLAU	
PLEK	SNCA	GJA7	
S100A8		RAGE	
S100A9		Unknown (image : 324383)	
S100A12		Unknown (image : 1411726)	
DSG2		Unknown (image : 1455566)	
		Unknown (image : 2012021)	

To explore the signature gene expression profiles over an infection period, we set the criteria that the change in expression level must be ≥ 1.5 or ≤ 0.667 fold at more than one time point. These criteria identified 131 up-regulated and 11 down-regulated genes that account for about 2% of the total 7,680 genes assessed. The differentially expressed genes were clustered hierarchically (Fig. **1**). The pattern revealed remarkable changes in gene expression, including both up- and down-regulation. Some genes were induced rapidly at T1 while others were induced at later times, most of the gene expressions returned to baseline level by T24. These results were presented in dendrograms in which the lengths of the branches are proportional to differences in gene expression of host at different time points.

These genes with a variety of biological functions represent a shared transcriptional response over the time course after infection. Their functions and biological processes involved were classified in Table **2**. A total of 8.5% (12/142) of the induced genes functionally belong to cytokines (IL1B, IL1RL1, G1P3, and PBEF1) or chemokines (IL8, MIP1B, CXCL1, 3, 13, and CCL2, 3, 20), which are important for cell growth and inflammatory responses. Notably, several other subsets of genes were identified with functions related to adhesion, cytoskeleton/motility, and calcium ion binding. The responsive genes which function in apoptosis or anti-apoptosis that were up-regulated included PRKR, PTEN, GADD45A, TNFAIP3, TNFAIP8, and SNCA. There were a large number of induced genes that were categorized as receptors or enzymes or have roles in signaling, transcription, and proliferation. We defined these 142 genes as the human monocyte signature genes that may reflect major signaling pathways or cascades stimulated upon the infection of *R. prowazekii*.

Defining Common Outliers and Most Abundantly Up Regulated Transcripts

Cluster analysis of expression profiles at these five time points indicated that those at T4 and T8, and T18 and T24 were more similar to each other by grouping in the same clusters, while that of T1 was the most dissimilar time point as illustrated by the dentrogram in Fig. (**2A**). Using the cut-off of ≥1.5-fold, we found six genes (ENG, GADD45A, TNFAIP3, IGFBP3, POU3F4, and ELK3) were affected over the entire infection time course. Therefore they were designated as common outliers. Their expression patterns are shown in Fig. (**2B**). These genes were up-regulated at T1 and T4. The fold of increase declined at T8, and then up again at T18. All of them returned to 1.5-2.5 fold induction at T24.

We also searched for highly induced genes among signature genes that together with common outliers might serve as biomarkers for *R. prowazekii* infection. We set a criterion for this selection: the induction of expression should be 2.0-fold or greater at more than one time point, or 3.0-fold or greater at a single time point. Twenty-two genes met this criterion (Table **3**). Among them, six genes are cytokines or chemokines (IL1B, MIP1B, CXCL1, CXCL3, CCL2, and CCL20); three are transcription factors (NFKB1A, ELK3, POU3F4); and the others as receptors, or genes involved in signaling, cell cycle, anti-apoptosis, and adhesion, etc.

IPA Pathway Analysis of Genes Affected at the Transcriptional Level After *R. prowazekii* Infection

To further investigate the biological pathways and interactive networks of these genes affected by rickettsial infection, IPA was used. IPA distributes selected genes into networks defined by known interactions from scientific publications and then associates these networks with biological

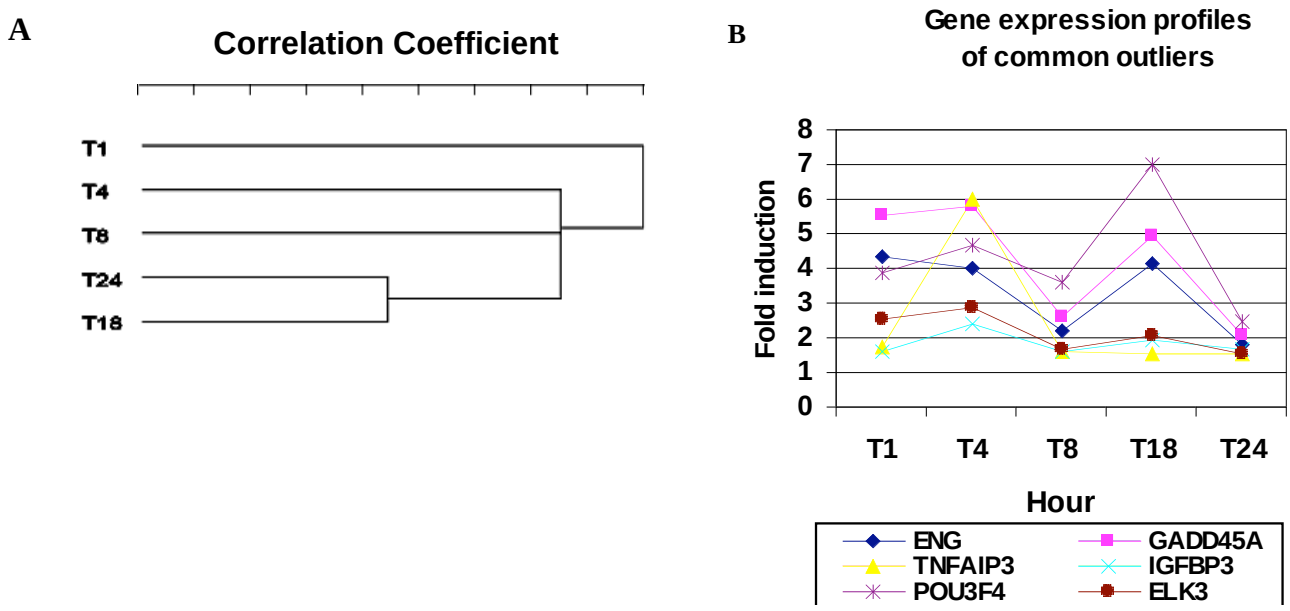

Fig. (2). The time course of gene response of THP-1 cells following *R. prowazkeii* infection. (**A**) Dendrogram comparing the correlation coefficient of host gene expression from T1-T24. (**B**) Graphical representation of the expression of common outliers through a time course.

Table 3. Twenty-Two THP-1 Genes Activated Post-Infection with *R. prowazekii* (≥2.0 in at Least Two Time Points or ≥3.0 at a Single Time Point)

Gene	T1	T4	T8	T18	T24
GADD45A	+	+	+	+	+
POU3F4	+	+	+	+	
ENG	+	+	+	+	
PPP1R14B	+	+		+	
ELK3	+	+		+	
CXCL1		+	+	+	
IL1B		+	+	+	
NFKB1A	+	+			
MIP1B/CCL4		+	+		
CXCL3		+	+		
CCL2		+	+		
TM4SF3		+	+		
TNFAIP6		+	+		
CCL20		+	+		
OLIG2		+		+	
CCNF		+		+	
RAE1		+		+	
LY64		+		+	
IM 1455566		+		+	
IM 2012021				+	+
TNFAIP3		+*			
TDO2				+*	

*>=3.0.

Table 4. Top 6 Networks Generated from IPA for Gene Expressions Affected by *R. prowazekii* Infection

ID	Genes*	Score	Top functions
1	**ACSL1, ACVR1, AMPD3,** BDKRB1, C9ORF26, CCBP2, **CCL2, CCL3, CCL4, CCL20, CD83,** **CITED2**, **COX8A, CREM, CXCL3, EGR2, GADD45A,** HAS1, **IGFBP3, IL8, IL1B, IL1RL1,** IRAK4, **NFKBIA,** PGDS, **PLAU,** PMP2, **PTEN, ROBO1, S100A8, S100A9,** SCYE1, SLC25A4, **TFAP2C, TNFAIP8**	46	Cellular Movement, Cell-To-Cell Signaling and Interaction, Viral Function
2	**ACADL,** ASS, **CA2,** CD82, CHST4, **CLIC4, CXCL13, EDNRB,** EIF2AK2, FABP1, FABP5, FPR1, **GADD45A,** HNRPA1, HSD11B1, HSP90B1, HSPD1, IFNK, IL24, **INDO,** ITGA4, ITGB7, **KYNU, LPXN, LTBP2**, NR1H3, NUTF2, PPARA, PPARD, **PTPN2, RAGE**, RETN, **RGS1,** ST6GAL1**,** TNF	21	Cardiovascular System Development and Function, Cellular Movement, Lipid Metabolism
3	ACVRL1, BRCA1, CAPN2, CAPN8, **CAPN9,** CAPN11, **CDH2,** CSH1, CTSD, **CYP1B1,** DDX5, **DSG2, DUSP6, EFEMP1,** EGF, ELF3, **ENG,** ESR1, **G1P3, GADD45A,** GJB1, **GOLGA4,** **HIST1H4C,** IRF6, IRS2, **PIK3R2,** PLSCR1, **PPP1R14B,** PTGS1, SERPINB5, **STC1,** TERT, TGFB3, TGFBR2, VEGF	19	Cancer, Cellular Movement, Drug Metabolism
4	**APOH, AQP4, ARL4A,** BMPR1B, CASP3, CCL9, CTNNB1, CYP2E1, DMD, DTX1, **ECT2,** GCG, GCLC, **KLRC3,** LEP, MARCKSL1, **OLIG2,** OSM, PCSK1, PCSK2, PIGA, PIGC, PIGP, PIGY, **POU3F4, PRG1,** SHH, SIM1, **SNCA,** ST8SIA5, **TDO2,** TNFRSF11B, TNFSF11, TSPAN8, **WBP1**	19	Cellular Development, Tissue Morphology, Skeletal and Muscular System Development and Function
5	**ABCA1**, **ARF5**, BIRC2, **CCL4**, CDKN1A, CTSS, CYBA, **CYBB**, DIABLO, G1P2, GADD45B, HGF, HNRPA2B1, IFNG, IL32, IRF2, KRT19, LDHA, **NCF4,** OCLN, **PDE4B**, PIM1, PLA2G1B, **PLA2R1**, **PLEK**, PRTN3, **PTPRB**, **RARG**, SCYE1, SLC2A1, SLC2A2, SPI1, ST8SIA1, **TFEC**, **TNFAIP6**	17	Cellular Growth and Proliferation, Cell Death, Immune and Lymphatic System Development and Function
6	APC, ARG1, **ARPC2,** ARPC5, CALM2, CDK2AP1, **CDR2, CYP2C9, DEAF1,** **DHRS9,** E2F1, **FCER1A,** FCGR1A, FKBP5, G3BP, GBP2, **GEM,** HSP90B1, IER3, **KLF2,** LDHA, MYC, NR3C1, **POLA2, PPL,** RHOB, ROCK1, ROCK2, **RPS15A**, S100A6, SERPINF2, SGK, **SPRR1B,** TGFB1, TGTP	15	Cancer, Cellular Growth and Proliferation, Endocrine System Disorders

*Bold genes were those identified by the microarray analysis (Bold only: up-regulated genes, bold with underline: down-regulated genes). Other genes were either not on the expression array or not significantly regulated. A score of >3 were considered statistically significant ($p<0.001$) by IPA.

pathways. IPA identified 10 statistically significant networks from 142 signature genes. The first six significant networks are shown in Table **4**. Results showed that there are statistically significant interactions among these gene products that were modulated by *R. prowazekii*. These networks involve cellular movement, cell- to -cell signaling and interaction, lipid metabolism, cellular growth and proliferation, cell death, immune response, etc. Fig. (**3**) showed the top first network identified by IPA. Among this network, CCL2, IL1B, IL8, and NFKB1A seemed to be located at the center points to connect multiple points, suggesting their crucial roles in response to *R. prowazekii*.

Confirmation of Responsive Genes by Real Time PCR

From the lists of the most highly induced genes and the common outliers, the expressions of six genes were selected for further analysis by Taqman quantitative real-time reverse transcriptive PCR (RT-qPCR). The same preparation of RNA used in microarray experiment was used for qPCR with primers and probes specific for GADD45A, PPP1R14B, ENG, NFKB1A, IL1B, CXCL1, and TNFAIP3. All these genes showed an induction in different magnitude over the time course (Table **5**). Generally speaking, the data from microarray screening and qPCR were in good agreement. The identification and confirmation of a core set of genes which were induced early in the rickettsial pathogenesis might be of value in the development of new strategies for early diagnosis.

DISCUSSION

The approach of global gene expression profiling on cDNA microarrays was employed and revealed a large number of genes differentially regulated. The results also suggested that the host response to rickettsial infection is a complicated process involving both induction and repression of groups of genes, with up-regulated genes the overwhelming majority. The genes induced by the infection were diverse in their functions as well. Overall, the alterations in the expression of these genes occurred in a time-dependent manner. Observing networks of differentially expressed genes during infection suggested that the co-expressed genes might also be co-regulated. The expression pattern in this study was compared with the features of eight other bacteria infections including Gram-negative, Gram-positive bacteria and mycobacteria [12]. This comparison revealed that about a quarter of 142 regulated genes caused by *R. prowazekii* infection were also reported in these infections. Majority of the genes identified in this study are not differentially expressed during other bacterial infections. It is reasonable to hypothesize that clusters of up- or down-regulated genes would constitute a signature pattern of expression, which might be specific for rickettsial infection (Table **2**).

To establish the list of genes that responded most substantially to the pathogen *R. prowazekii*, a total of 22 host genes were identified (Table **3**). Among them, the inductions of eight genes were detected at three or four time points (GADD45A, POU3F4, ENG, PPP1R14B, ELK3, CXCL1,

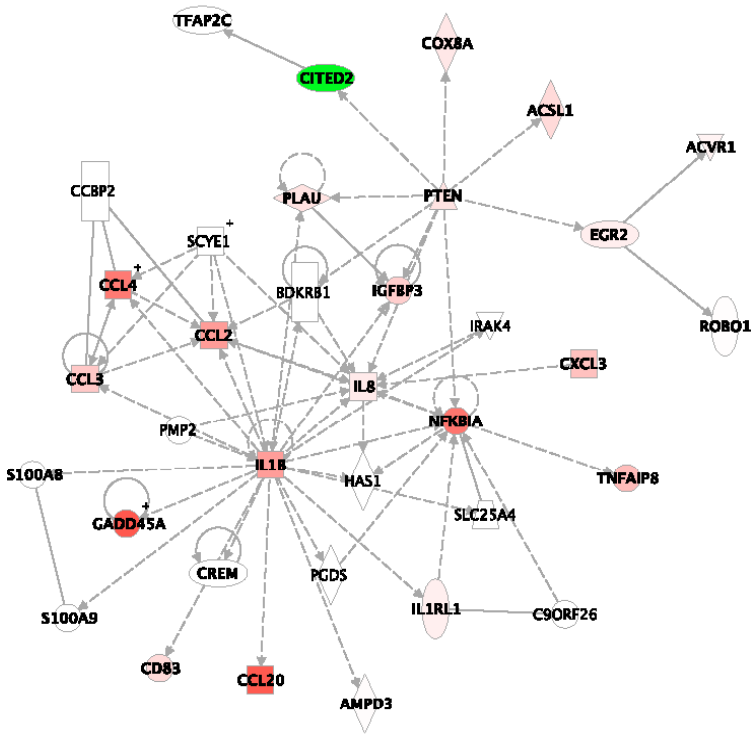

Fig. (3). A representative of networks of genes regulated after *R. prowazekii* infection. Twenty-five genes that were up-regulated (Red, the darker the higher induction) and one gene that was down-regulated (Green) in THP-1 cells were analyzed by Ingenuity Pathway Analysis (IPA) software. Other genes are those associated with the regulated genes.

Table 5. Quantitative Real-Time PCR for Selected Host Response Genes

Gene	C_T [a] (X± SD) [b] and [c]Fold Expression Change									
	T1		T4		T8		T18		T24	
18S rRNA [d]	26.16 ± 2.41 25.71 ± 0.20		29.86 ± 0.23 23.64 ± 0.19		23.20 ± 0.10 21.32 ± 0.20		27.03 ± 0.24 25.88 ± 0.19		22.42 ± 0.19 23.58 ± 0.18	
GADD45A	32.96 ± 0.44 33.40 ± 0.20	1.88	36.20 ± 0.37 32.62 ± 0.63	6.25	32.53 ± 0.26 32.43 ± 0.20	3.43	34.96 ± 0.19 36.05 ± 0.40	4.63	34.60 ± 0.71 34.14 ± 0.58	2.17
PPP1R14 B	36.26 ± 1.36 36.32 ± 1.46	1.42	38.95 ± 1.37 33.83 ± 0.29	2.17	34.40 ± 0.78 34.21 ± 0.29	3.23	36.22 ± 0.69 37.40 ± 0.18	5.01	33.60 ± 0.35 33.63 ± 0.24	2.28
ENG	33.64 ± 1.21 33.09 ± 0.30	0.93	37.38 ± 0.98 32.48 ± 0.40	2.50	31.56 ± 0.02 32.10 ± 1.13	5.35	33.56 ± 0.35 33.34 ± 0.37	1.91	31.50 ± 0.38 31.28 ± 0.15	1.92
NFKB1A	26.15 ± 0.07 26.63 ± 0.08	1.91	27.38 ± 0.22 27.22 ± 0.10	66.72	26.11 ± 0.15 25.78 ± 0.15	2.93	26.47 ± 0.08 27.36 ± 0.23	4.11	25.45 ± 0.17 26.00 ± 0.06	0.73
IL1B	33.27 ± 0.68 32.82 ± 0.22	1.00	33.75 ± 0.09 31.27 ± 0.31	13.36	32.43 ± 0.27 34.18 ± 0.22	12.38	30.29 ± 0.19 30.37 ± 0.11	2.35	30.75 ± 0.66 32.12 ± 0.37	1.29
CXCL1	32.05 ± 0.34 31.75 ± 0.38	1.11	33.22 ± 0.36 32.35 ± 0.67	40.79	30.83 ± 0.38 33.35 ± 0.13	21.11	30.32 ± 0.26 31.22 ± 0.29	4.14	28.68 ± 0.39 30.03 ± 0.21	1.27
TNFAIP3	36.31 ± 0.61 35.95 ± 0.30	1.06	37.18 ± 0.15 37.31 ± 0.88	81.57	36.38 ± 0.07 37.90 ± 0.42	10.56	37.87 ± 0.19 39.18 ± 0.13	5.50	35.60 ± 0.81 36.37 ± 0.46	0.85

[a] C_T represents the cycle number at which a significant increase in fluorescence signal above a threshold signal (horizontal zero line) can first be detected.
[b] X and SD, average and standard deviation values.
[c] Using the comparative ($\Delta\Delta C_T$) method.
[d] Internal control.

IL1B, and NFKB1A) and others were seen at two time points. Six out of 22 genes belong to the category of cytokine or chemokine families, which are commonly seen in other infectious diseases. However, other genes are not commonly up-regulated due to microorganism infections and thus were not previously suspected of being transcriptionally regulated by rickettsial infection. Previous studies on these genes such as POU3F4 [13], PPP1R14B, TM4SF3 [14], OLIG2 [15], RAE1 [16], LY64 [17], and TDO2 [18] are very limited. For example, ENG, which plays a role in adhesion and receptor binding, was up-regulated in this study but was down-regulated in other infectious events including Gram positive and Gram negative bacteria [9]. POU3F4 (Brain-4) is a transcription factor mainly expressed in pancreatic cells [10] but its role in disease is unknown. In addition, six common outliers (ENG, GADD45A, TNFAIP3, IGFBP3, POU3F4, ELK3) that were up-regulated in the entire time course were identified. Five of them (ENG, POU3F4, TNFAIP3, GADD45A and ELK3) were also among the highly stimulated group.

Although we did not observe significant induction of IFNγ which is usually up-regulated in rickettsial infection, we did notice the induction at T8 (1.2919). There was no increase at T1 (1.0042) and T24 (0.8695). Unfortunately, we excluded the IFNγ data from T4 and T18 for analysis due to "bad spots". Therefore, the status of IFNγ in this study is not fully clear. We also saw increases of IFNγ receptor 2 at T1 (1.3512) and T4 (1.9655) in this study.

By using IPA, the interactive networks of genes were constructed. IPA identified statistically significant genes that participate in the multiple pathways with mutual interactions. Further functional analysis of these associated networks and pathways as well as dose response and protein level may provide additional insight into the macrophage defense response against *R. prowazekii* infection.

CONCLUSION

Our findings have uncovered the molecular features of host responses to *R. prowazekii* infection at genome-wide level. In addition, this study may pave the road for further studies of interaction between rickettsia and host.

ACKNOWLEDGMENTS

This work was supported by the Naval Medical Research and Development Command, Work Unit 6000.RAD1.J. A0310. The views expressed in this article are those of the author and do not necessarily reflect the official policy or position of the Department of the Navy, Department of Defense, nor the U.S. Government. We would like to thank Lieutenant Paul Graf for comments and suggestions of this manuscript.

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Received: December 31, 2007 Accepted: April 15, 2008

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