INFECTION AND IMMUNITY, Jan. 2004, p. 498–507 Vol. 72, No. 1
0019-9567/04/$08.00+0 DOI: 10.1128/IAI.72.1.498–507.2004

Survival Strategy of Obligately Intracellular *Ehrlichia chaffeensis*: Novel Modulation of Immune Response and Host Cell Cycles

Jian-zhi Zhang,[1] Mala Sinha,[2] Bruce A. Luxon,[2] and Xue-jie Yu[1]*

Departments of Pathology and Microbiology and Immunology[1] and UTMB Bioinformatics Program and the Department of Human Biological Chemistry and Genetics,[2] The University of Texas Medical Branch, Galveston, Texas 77555

Received 1 July 2003/Returned for modification 12 September 2003/Accepted 29 September 2003

***Ehrlichia chaffeensis* is an obligatory intracellular bacterium which resides in an early endosome in monocytes. *E. chaffeensis* infection in a human monocyte cell line (THP1) significantly altered the transcriptional levels of 4.5% of host genes, including those coding for apoptosis inhibitors, proteins regulating cell differentiation, signal transduction, proinflammatory cytokines, biosynthetic and metabolic proteins, and membrane trafficking proteins. The transcriptional profile of the host cell revealed key themes in the pathogenesis of *Ehrlichia*. First, *E. chaffeensis* avoided stimulation of or repressed the transcription of cytokines involved in the early innate immune response and cell-mediated immune response to intracellular microbes, such as the interleukin-12 (IL-12), IL-15, and IL-18 genes, which might make *Ehrlichia* a stealth organism for the macrophage. Second, *E. chaffeensis* up-regulated NF-κB and apoptosis inhibitors and differentially regulated cell cyclins and CDK expression, which may enhance host cell survival. Third, *E. chaffeensis* also inhibited the gene transcription of RAB5A, SNAP23, and STX16, which are involved in membrane trafficking. By comparing the transcriptional response of macrophages infected with other bacteria and that of macrophages infected with *E. chaffeensis*, we have identified few genes that are commonly induced and no commonly repressed genes. These results illustrate the stereotyped macrophage response to other pathogens, in contrast with the novel host response to obligate intracellular *Ehrlichia*, whose survival depends entirely on a long evolutionary process of outmaneuvering macrophages.**

Ehrlichia chaffeensis is a gram-negative obligate intracellular bacterium which resides in a vacuole within host cells (6, 13). It causes human monocytic ehrlichiosis, an emerging infectious disease first reported in 1987. Human monocytic ehrlichiosis is a moderate to severe disease, with a case fatality rate of approximately 3% (27). The life cycle of *E. chaffeensis* includes a mammalian host and a tick vector (3). *E. chaffeensis* is transmitted in ticks transstadially but not transovarially. To overcome this lack of efficient maintenance in ticks, *E. chaffeensis* has evolved to establish persistent infection in its natural animal hosts, such as white-tailed deer (12) and canines (14). The principal target cell of *E. chaffeensis* is the monocyte/macrophage lineage. The tropism of this organism for monocytes and its ability to evade normal phagocytic pathways suggest that the organism may have evolved for some unique pathways for intracellular survival and development of infection. *E. chaffeensis* resides in an early endosome. The survival of *E. chaffeensis* inside the host cell depends on inhibiting fusion of the phagosome and the lysosome (6).

Understanding the transcriptional profiles of monocyte genes at various time points in response to *E. chaffeensis* will help us to decipher the tactics used by *E. chaffeensis* to evade host cell responses and thus will aid future efforts in developing therapeutics. For this study, we used the HG-U95Av2 gene chip (Affymetrix Inc., Santa Clara, Calif.), containing 12,599 sequenced human genes or expressed sequence tags (ESTs), to measure gene expression profiles of the THP1 monocyte cell line 1, 7, 11, and 24 h after exposure to *E. chaffeensis* (38). We provide some insight into the mechanisms used by *E. chaffeensis* to block fusion of the phagosome and the lysosome, to evade the host immune system, and to inhibit host cell apoptosis and enhance host cell survival, which are essential to the well-being of the ehrlichiae.

* Corresponding author. Mailing address: Department of Pathology, University of Texas Medical Branch, 301 University Blvd., Route 0609, Galveston, TX 77555-0609. Phone: (409) 747-1786. Fax: (409) 747-2415. E-mail: xuyu@utmb.edu.

MATERIALS AND METHODS

***E. chaffeensis*.** *E. chaffeensis* strain Arkansas was cultivated in THP1 cells, a human monocyte cell line, with 10% bovine calf serum-supplemented Dulbecco's modified Eagle medium at 37°C. When 90% of the cells were infected (at approximately 5 days postinfection), ehrlichiae were harvested. The cells were centrifuged for 20 min at 12,100 × *g*. The pellet was suspended in SPK buffer (0.2 M sucrose, 0.05 M potassium phosphate buffer, pH 7.4) (35) and sonicated twice for 10 s on ice at 40 W, using an Ultrasonic processor (Sonic & Materials Inc., Newtown, Conn.). The suspension was centrifuged at 200 × *g* for 10 min to remove cell debris. The supernatant was centrifuged for 20 min at 12,100 × *g*. The pellet was suspended in freezing medium (10% dimethyl sulfoxide, 20% bovine serum, and 70% minimal essential medium). The ehrlichial suspension was divided into aliquots and stored at −80°C as a stock for subsequent infection of THP1 cells and determination of the *E. chaffeensis* infectious content.

The *E. chaffeensis* infectivity titer was determined by limiting dilution of host cell-free ehrlichiae. Briefly, diluted ehrlichiae were applied onto DH82 cell monolayers in 24-well plates and incubated at 37°C with 5% CO_2 for 14 days, with a medium change every 3 days. On days 7 and 14 after infection, cells were examined by PCR and Diff-Quik staining for *E. chaffeensis* infection. DNA was extracted from cells by use of a Qiagen DNA blood mini kit (Qiagen, Valencia, Calif.). One microliter of DNA was used to amplify the *gp120* gene of *E. chaffeensis* with primers pxcf3b (5′-CAG CAA GAG CAA GAA GAT GAC) and pxar5 (5′-ATC TTT CTC TAC AAC AAC CGG) (39). PCR amplification was performed for 30 cycles of 94°C for 40 s, 55°C for 40 s, and 72°C for 1 min, with a final extension of 7 min. The size of the PCR product was verified by agarose gel electrophoresis. For Diff-Quik staining, 200 μl of supernatant from each well was centrifuged onto a slide with a Cytospin centrifuge. The slides were stained and examined for *E. chaffeensis* morulae.

***E. chaffeensis* DNA and RNA isolation.** THP1 cells (2×10^6 cells/ml) were infected with host cell-free *E. chaffeensis* at a multiplicity of infection of 100 and cultivated at 37°C under the same conditions as those described above. Samples of THP1 cells (50 ml) were obtained at 1, 7, 11, and 24 h postinoculation and used for DNA and total RNA isolation by use of a Qiagen DNA blood mini kit (Qiagen) and NucleoSpin RNA and virus purification kits (Clontech), respectively. THP1 cells (50 ml) taken prior to inoculation of *E. chaffeensis* were used as a 0-h time point control. One microliter of DNA was used to amplify the *gp120* gene with primers pxcf3b and pxar5 to confirm *E. chaffeensis* infection. The purity of the RNA was determined by use of the PicoGreen RNA quantitation kit (Molecular Probes). The integrity of RNA was verified by agarose gel electrophoresis.

cDNA target preparation and array hybridization. The HG-U95Av2 gene chip (Affymetrix Inc.), containing 12,599 sequenced human genes and ESTs, was used for screening gene expression. First-strand cDNA synthesis was performed with total RNA (10 to 25 μg), a T7-$(dT)_{24}$ oligomer (5′-GGCCAGTGAATTGTAA TACGACTCACTATAGGGAGGCGG-dT_{24}-3′), and SuperScript II reverse transcriptase (Life Technologies). Second-strand synthesis converted the cDNA into a double-stranded DNA template for use in an in vitro transcription reaction. The T7 promoter introduced during first-strand cDNA synthesis provided the necessary sequence for directing the synthesis of cRNA with bacteriophage T7 RNA polymerase. The cRNA targets were labeled with biotin during the in vitro transcription reaction. cRNAs labeled with biotin were fragmented to a mean size of 200 bases to facilitate their hybridization to probe sequences on the gene chip array. Each target sample was initially hybridized to a test array to confirm the successful labeling of the cRNAs and to prevent the use of degraded or nonrepresentative target cRNA samples. The test array contained a set of probes representing genes that are commonly expressed in the majority of cells (actin, glyceraldehyde-3-phosphate dehydrogenase [GAPDH], transferrin receptor, transcription factor ISGF-3, 18S rRNA, 28S rRNA, and *Alu* genes).

Hybridization of the HG-U95Av2 gene chip arrays was performed at 45°C for 16 h in hybridization buffer (0.1 M morpholineethanesulfonic acid [pH 6.6], 1 M NaCl, 0.02 M EDTA, and 0.01% Tween 20). Four prokaryotic genes (*bioB*, *bioC*, and *bioD* from the *Escherichia coli* biotin synthesis pathway and *cre*, the recombinase gene from bacteriophage P1) were added to the hybridization cocktail as internal controls. Arrays were washed under both nonstringent (1 M NaCl, 25°C) and stringent (1 M NaCl, 50°C) conditions prior to staining with phycoerythrin-streptavidin (final concentration, 10 μg/ml).

Gene chip arrays were scanned with a Gene Array scanner (Hewlett-Packard) and analyzed with Gene Chip Analysis, suite 3.3, software (Affymetrix Inc.). For each gene, 16 to 20 probe pairs were immobilized as ~25-mer oligonucleotides that hybridized throughout the mRNA; each probe pair is represented as a perfect match (PM) oligonucleotide and a mismatch (MM) oligonucleotide used as a hybridization control. The average intensity of each probe cell was calculated after subtraction of the local background (the lowest 2% intensity of each sector; each probe cell is divided into 16 sectors). The normalized average intensity value was used to determine the number of positive and negative probe pairs. Based on the positive/negative ratio, the positive fraction, and the log average ratio of the PM to the MM, the absolute call (i.e., expression of the gene is detected [present] or not [absent]) was determined. Finally, the average difference was determined by calculating the difference in intensity between the PM and the MM of every probe pair and averaging the differences over the entire probe set. The average difference statistic was retrieved for quantification of mRNA abundance for those samples in which the absolute call indicated that the gene was present. Probe set data were deposited into our data warehouse and relational database server (http://www.bioinfo.utmb.edu).

Data analysis for oligonucleotide probe-based microarrays. Following normalization against the housekeeping genes, the oligonucleotide spot intensity values for each array were compared for the different time points. Gene probe sets with an absolute call of "absent" across all the chips were removed, and gene probe sets that changed $\geq|3|$-fold in any one of the possible pairwise comparisons were used for further analysis of *K* means and hierarchical cluster analysis, using the software package Spotfire (Somerville, Mass.).

To more reliably profile global changes in gene expression, we analyzed the reproducibility of the data generated for two independent time courses by log-log plots of the average differences in signal for the independently performed arrays for each time point of *E. chaffeensis* infection. At each time point, linear regression analysis was performed. In addition, a hierarchical clustering algorithm was used to analyze the reproducibility of the data generated in different arrays in independent time courses.

Confirmation of differential expression of genes. The expression of selected genes was further analyzed for *E. chaffeensis*-infected THP1 cells by reverse transcription (RT)-PCR. Total cellular RNA (2 μg) was used for first-strand cDNA synthesis with the RETROscript first-strand synthesis kit (Ambion Inc., Austin, Tex.). PCRs were carried out by use of the Roche PCR master kit (Roche Biochemicals, Indianapolis, Ind.). PCR conditions were the same as those described above. Two negative controls, including a −RT control without reverse transcriptase and a minus-template PCR without sample cDNA, and one positive control, the control template RNA from the kit, were used in PCRs to verify the RT-PCR. The selected host genes and their primers are listed in Table 1.

RESULTS

Evaluation of the reproducibility and reliability of the oligonucleotide probe-based microarray experiments. Oligonucleotide array hybridization reactions were performed twice on two separate occasions with RNA prepared from THP1 cells infected with *E. chaffeensis*. Genes with expression levels that changed in response to *E. chaffeensis* were selected on the basis of repeated differences in the expression levels of the treated and untreated samples across multiple time points. The data from two independent experiments were tightly clustered within $|2|$-fold changes, as determined by linear aggregation analysis. At each time point, linear regression analysis showed a slope of 0.909, with a Pearson correlation coefficient of >0.9 for each pairwise comparison (Fig. 1). This indicates that the data were linear and that data points from the two experiments were highly reproducible.

Hierarchical clustering algorithm analysis showed that the data generated in different arrays in independent time courses were tightly clustered at different time points and further confirmed the reproducibility of the results (data not shown).

The oligonucleotide probe-based microarrays used here contain several multiple probe sets, with oligonucleotides complementary to the same mRNAs. Altered expression of L24564, a Ras-related gene associated with diabetes, and U22376, a v-Myb myeloblastosis viral oncogene homolog (avian), were each confirmed by consistent results from several independent probe sets targeted to different regions of their mRNAs. Several cases produced different results from different probe sets, which could reflect false positives, alternative mRNA splicing, or the different specificities and cross-hybridization possible with different probe sets.

Confirmation of gene transcription by RT-PCR. RT-PCR amplification of monocyte transcripts was positive for interleukin-8 (IL-8), human apoptosis-related protein TFAR15, and E-selectin and L-selectin ligand sulfotransferase but was negative for human inhibitor of apoptosis protein 1 (Hiap1), IL-1α, IL-4, IL-6, IL-12α, IL-12β, IL-15, and IL-18. The results were consistent with the microarray results and confirmed the accuracy of our microarray data (Table 1).

Screening of oligonucleotide probe-based microarrays. Biotin-labeled target cDNAs prepared from total RNA extracted from THP1 cells following exposure to *E. chaffeensis* for 1, 7, 11, and 24 h and uninfected control THP1 cells were hybridized to the HG-U95Av2 gene chip, containing 12,599 sequenced human genes. We plotted the number of genes whose expression changed by a factor of 3 (conservatively chosen to minimize the number of false positives) relative to that in uninfected cells. Of the 12,599 genes represented on the oligonucleotide array, 903 tested genes or ESTs were found to have a significant change ($\geq|3|$-fold) in at least one of the comparisons during the 24-h infection period, corresponding

TABLE 1. Confirmation of the oligonucleotide array results by RT-PCR amplification of selected genes

Gene	Primer[b]	Fold change in transcription level in oligonucleotide array at h[a]:			Confirmation by RT-PCR at h:		
		1	11	24	1	11	24
Hiap1	TGACCTTGTCATTCACACCAG GCCATTAAATGGCATCCTGAT	1.2	7.3	5.1	−	+	+
IL-1a	CAAGGAGAGCATGGTGGTAGTAGCAACCAACG TAGTGCCGTGAGTTTCCCAGAAGAAGAGGAGG	−0.3	−0.5	−0.8	−	−	−
IL-4	CTCATGATCGTCTTTAGCCT CTCTGTTCTTCCTGCTAGCAT	−1.2	−0.8	−0.6	−	−	−
IL-6	AATTCGGTACATCCTCGACGG TGACCAGAAGAAGGAATGCCC	−0.8	−0.8	−0.9	−	−	−
IL-8	AAGAGCCAGGAAGAAACCACC ATTGCATCTGGCAACCCTACA	8.5	2.5	1.0	+	+	+
IL-12a	CCGGCTCAGCATGTGTCCA CAGCTCATCAATAACTGCCAGCA	UD	UD	UD	−	−	−
IL-12b	GATGGTATCACCTGGACCTTG GCATCCACCATGACCTCAAT	UD	UD	UD	−	−	−
IL-15	GATGGATGGCTGCTGGAA CATTGCTGTTACTTTGCAACTG	−1.1	−7.0	−1.5	−	−	−
IL-18	CTCAGACCTTCCAGATCGCTT GTGATCTGCCCGCCTCAG	−2.5	−4.6	−2.0	−	−	−
E-selectin	CCGAAGGGTTTGGTGAGGTGTGCT AAATGGTGCTAATGTCAGGAGGGAGAGTC	2.4	1.2	1.1	+	+	+
P-selectin	ACTGGGCTGATAATGAACCTAACAACAAAA GAGGCTTATTTGTCCAGATTCCAGA	UD	UD	UD	−	−	−
SELPLG	CCACCAGCAGCCACGGAAGC GCCACCAGCGCCAAGATTAGGAT	1.23	1.4	1.2	+	+	+
TFAR15	GATGAATTAGTCGGTTGGCAC GAACGAGTAAATCTGTCTGCAG	−1.4	1.3	1.3	−	+	+

[a] Fold change in macrophage gene transcriptional level 1, 11, and 24 h after *E. chaffeensis* infection compared to the transcriptional level before infection (0 h). UD, signal below detectable level.
[b] For each set, the first primer is the forward primer and the second primer is the reverse primer.

to 7.2% (903 of 12,599) of the genes on the chip. After subtraction of the genes whose transcripts were detected at more than one time point of a single experiment, 570 genes of the monocytes had significantly changed transcriptional levels for at least one time point after *E. chaffeensis* infection, which is 4.5% of the total number of genes tested (Fig. 2; Tables 2 and 3). The numbers of genes with altered expression (induced/repressed) at 1, 7, 11, and 24 h were 284 (140/144), 236 (151/85), 218 (129/89), and 173 (101/72), respectively. The number of genes with a changed transcriptional level decreased while the infection was progressing. At the earliest time point (1 h), the number of genes that were upregulated was approximately equal to the number of genes that were downregulated. At the middle (7 and 11 h) and late (24 h) time points, upregulated genes predominated.

We further analyzed the data by classifying threefold-regulated genes by their primary functions. Although no single biochemical process could be identified, the profiles of host cell gene transcripts included those for proteins inhibiting apoptosis and regulating cell differentiation, signal transduction and transcription factors, proinflammatory cytokines, biosynthesis and metabolism, membrane trafficking, adhesion, and structure. The transcription of genes related to the immune response to *E. chaffeensis* infection and intracellular survival of *E. chaffeensis* was particularly interesting and is described in detail below.

Immune response to *E. chaffeensis* infection. At the early and middle stages of infection (1 to 7 h), *E. chaffeensis* induced transcription of monocyte genes for IL-1β, IL-8, and tumor necrosis factor beta (TNF-β). Transcription of monocyte genes for small inducible cytokines such as A3, A4, A5 (RANTES), and Cys-Cys member 20 was induced 1 to 11 h after infection, and A4 transcription was induced at all time points.

E. chaffeensis repressed monocyte gene transcription of IL-15, IL-18 (Fig. 3), and small inducible cytokine subfamily A (Cys-Cys) member 23. The transcription of IL-10 and IL-12 in monocytes was not changed after *E. chaffeensis* infection.

Cytokine receptors were generally repressed by *E. chaffeensis* infection. These receptors included chemokine (C-C motif) receptors 2, 3, and 4, IL-8 receptor, and IL-13 receptor. IL-7 receptor was the only cytokine receptor of monocytes that was induced by *E. chaffeensis*.

Membrane trafficking. Molecules mediating vesicle docking were generally repressed by *E. chaffeensis*. *E. chaffeensis* repressed the transcription of SNAP23 (synaptosomal-associated protein; 23 kDa), Rab5A (member of RAS oncogene family), and STX16 (syntaxin 16) significantly ($>|3|$-fold) during early infection (1 h). The genes for these proteins were also repressed at the later time points, but to a lesser extent (twofold). Vimentin, a reservoir for SNAP23, was induced 1 to 7 h after *E. chaffeensis* infection (Fig. 4).

Apoptosis. Apoptosis inhibitors were generally induced by *E. chaffeensis* infection of monocytes. NF-κB (NFKBIA) gene transcription was induced in monocytes at all time points after *E. chaffeensis* infection. Apoptosis inhibitor IER3 (immediately early response 3) was induced significantly at all time points, with a peak 1 h after infection. BirC3 (baculoviral IAP repeat-containing protein 3) was significantly induced from 7 to 24 h,

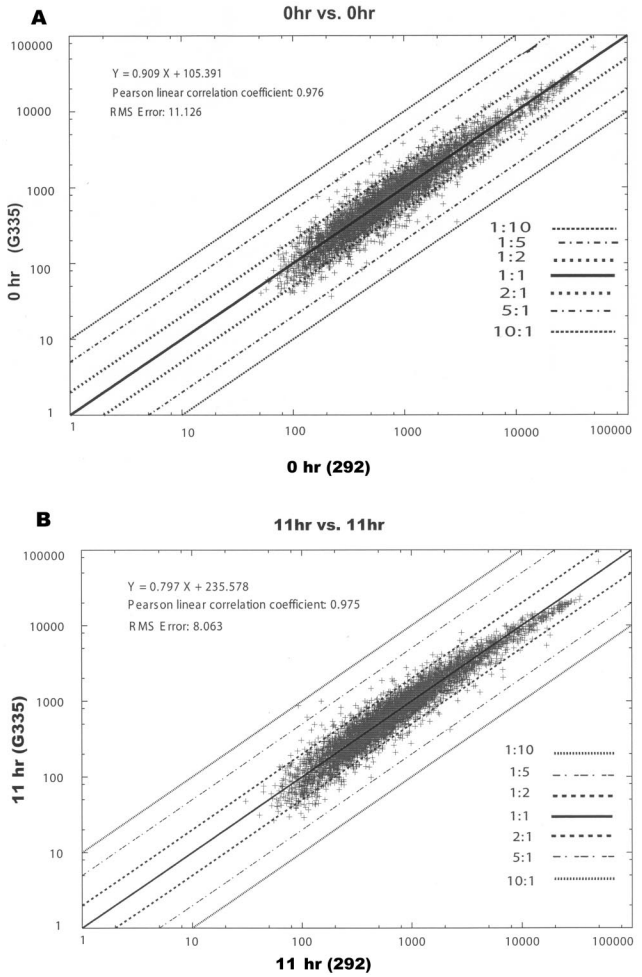

FIG. 1. Reproducibility of the oligonucleotide array. The average differences for the data at 0 h (A) and 11 h (B) from experiment 1 versus the corresponding time course for experiment 2 were plotted by pairwise comparisons. The only criterion for inclusion was that the probe set was designated "present" in both time series. Least-square linear regression was used to determine the fit to a straight line. For the 0-h data set, the regression was described by the equation $y = 1.172x - 715.408$ ($r^2 = 0.935$), and for the 36-h data set, the equation was $y = 1.198x - 741.671$ ($r^2 = 0.911$).

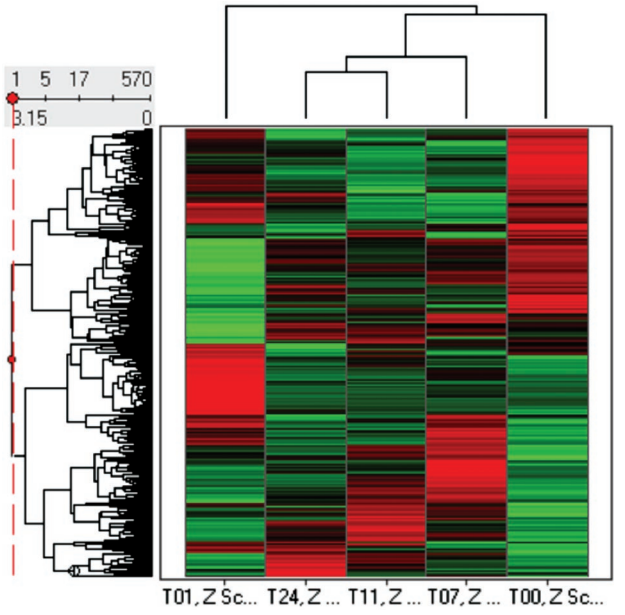

FIG. 2. Hierarchical cluster analysis of 570 genes with threefold changes after exposure to *E. chaffeensis*. T00, T01, T11, and T24 represented 0, 1, 7, 11, and 24 h postinoculation. Z-score values are displayed colorimetrically from top to bottom. Line lengths in the dendrogram indicate the correlation of the genes, with shorter lines indicating higher levels of correlation. Genes induced by *E. chaffeensis* are indicated in red, and genes with reduced expression are indicated in green. The degree of redness represents the level of induction, whereas that of greenness represents the level of repression. Each column presents the expression of that gene at the indicated time point relative to uninfected THP1 cells. The complete data set was deposited at http://www.bioinfo.utmb.edu.

with a peak at 7 h, after *E. chaffeensis* infection. The BCL2 (B-cell lymphoma 2) and BCL2-related proteins (MCL1 and BCL2A1) were differentially transcribed. MCL1 was induced in the first hour, and BCL2A1 was induced at 7 h. BCL2 was repressed at 7 h. Apoptosis inducers such as BIK (BCL2-interacting killer) and BNIP3L (BCL2/adenovirus E1B 19-kDa interacting protein 3-like) were downregulated at 7 h (Fig. 5). The transcription of caspase genes was not changed in monocytes after *E. chaffeensis* infection. Apoptosis inducer hematopoietic cell kinase (HCK) was upregulated from 7 to 24 h, peaking at 11 h.

Signal transduction and cell proliferation. *E. chaffeensis* downregulated many protein kinases. *E. chaffeensis* inhibits TXK (a tyrosine kinase), ITK (IL-2-inducible T-cell kinase), and RET transcription at all the time points studied during infection. Three p21-activated kinase genes (PAK1, -2, and -7) and STK4 (serine/threonine protein kinase Krs-2) were repressed 1 h after exposure to *E. chaffeensis.* CNK (cytokine-inducible kinase) was induced 1 h after exposure to *E. chaffeensis* but was repressed at other time points. Both JAK1 and STAT1 were downregulated during the first hour after *E. chaffeensis* infection. EPHA2 and DRT (developmentally regulated EPH-related tyrosine kinase) were induced at the earliest stage (1 h) and repressed at later stages (7, 11, and 24 h) of infection.

Many genes involved in controlling cell cycles in monocytes changed expression levels during *E. chaffeensis* infection. In the first hour postinfection, *E. chaffeensis* downregulated CDC2 (cell division cycle 2), CDK5 (cyclin-dependent kinase 5), CDK8, and cyclin G1. From 7 to 24 h postinfection, *E. chaffeensis* upregulated cyclin E1, cyclin E2, and CDC25 (Fig. 6).

DISCUSSION

We have analyzed the global gene transcriptional profile of human monocytes in response to *E. chaffeensis* infection by using oligonucleotide arrays. Our data provide evidence of differential expression of monocyte genes 1, 7, 11, and 24 h after infection with *E. chaffeensis*. *E. chaffeensis* infection altered the transcription of a wide range of genes across the host genome (4.5%), despite the fact that *E. chaffeensis* develops exclusively inside a vacuolar inclusion separated from the cy-

TABLE 2. Macrophage gene transcription induced by *E. chaffeensis* infection

Functional category or gene
Adhesion and structure
CD58
EMP2
EMP3
EPB41L3
ICAM1
PRPH
VIM
Apoptosis and cell proliferation
AJ011981
ANXA1
ARHGEF12
BCL2A1
BIRC3
BTG2
CCNE1
CCNE2
CD83
CDC25A
CDC6
CRIP1
CTGF
CYBB
CYP1B1
DNAJB1
DSPG3
EGR2
EPS8
FGF7
FTH1
HAS6591
HSPA1A
HSPA6
IER3
IGFBP3
LARGE
MCL1
MDM2
NCF1
NFKBIA
PLA2G7
PTGS2
PTTG2
PTX3
RAB3IP3
RELB
RGS2
RRAD
SERPINE1
SOD2
SPAG6
STATH
STC2
TNFAIP6
TNFRSF10B
TSSC3
TUB
Immune response
AF070578[a]
CD48
CORT
CYR61
GRO1
GRO2
HUMRTVLH3
ID2
IFI30
IGSF4
IL1B
IL7R
IL8
M59830
NAF1
NPTX1
PEA15
SCYA2
SCYA2
SCYA20
SCYA4
SCYA5
SIX6
TNF
TNFAIP2
TNFAIP3
TNFRSF9
TRAF3
Signal trasduction
ACVR2
ADORA2B
AHR
C3AR1
CNK
CYP27B1
DUSP10
DUSP14
DUSP8
EBI2
EPHA2
EPHB2
F3
FGR
FZD7
GP1BA
GPR51
HCK
HG3484-HT3678
HSD11B1
HTR1E
KCNAB1
KCNN3
KCNN4
MD-2
NPR3
NR4A1
PIM1
PIM2
PPM1A
PROCR
SCYA3
SGK
WSX1
DNA and protein binding
ANXA8
ARHE
CHAF1B
CTNNBIP1
GEM
ITIH2
LGALS3
MARCKS
NEBL
RAB36
RIN1
RPGR
RPP14
S100A10
SYT1
TMPO
WARS
Oncogene
ERF
EXT1
RB1
FOSB[a]
Transporter
ATP2B1
SLC11A2
SLC17A4
SLC31A1
SLC7A7
Transcription
CHD1L
CRIP2
DSCR1
E2F6
EGR3
ETR101
ETV5
ETV6
GAS7
JUN
JUNB
JUND
LOC51042
MAF
MAFF
MEOX2
MRPS10
MSC
MTF1
MYBL1
NAB2
NFE2L2
NFE2L3
NFKB1
NFKB2
NFKBIE
NMP200
NR4A2[a]
OLIG2
PCNA
PIR
PLAG1
TFAP2A
ZFP36
ZNF140
ZNF202
ZNF297B
Metabolism
ADAM17
ADAM28
ALAS1
ALDH1A1
ANXA2
AQP9
ASAH
CHST2
CTSH
CTSZ
DPYD
DTYMK
ECGF1
FACL2
FADS3
FUT3
GCH1
GGH
IDS
ME1
MGAT2
MMP9
PDE5A
PLA2G4C
SAT
SLC1A5
TJP2
TPS1
UGCG
UP
Miscellaneous
AA534868
AD024
ADAMDEC1
ADFP
AI432401
ATIP1
BCL3
BTG3
CAPRI
COPEB
DRD1
FGL2
GPNMB
HG172-HT3924
HRB2
HRY[a]
J04755
JTB
KPNA5
LHX2
LOC55884
LPXN
M59287
M62895
MAPRE3
OPTN
P8
PER2
PRAX-1
QPCT
SCEL
SCO2
SDC4
V01512
VLGR1
XRCC3
ESTs
AF027153
AF038174
AL049265
AL080190
DKFZP434J214
DKFZP564D0462
DKFZP566B183
DKFZp586G0123
FLJ10803
FLRT2
KIAA0186
KIAA0189
KIAA0379
KIAA0410
KIAA0429
KIAA0507
KIAA0690
KIAA0951
KIAA1564
PSORT
Rab11-FIP2
W26472

[a] Transcription of the genes was induced and suppressed at different time points.

TABLE 3. Macrophage gene transcription repressed by *E. chaffeensis* infection

Functional category or gene
Adhesion and structure
ACTA1
ADARB1
ADD3
CDH18
COL4A1
COL4A5
COL9A3
CTNNA1
DMD
DSC2
ITGA4
LAMA4
SELE
SPTBN1
TGFA
THBS4
TUBA1
Apoptosis and cell proliferation
AP15
ATP11A
BCL2
BIK
BMP4
BNIP3L
CDC14B
CDK5
CDK8
CIS4
CORO2A
CSPG2
DEFB1
FLNA
FMO5
GAGE1
IGFBP2
IGFBP4
KEO4
LOC58509
LSP1
MASP1
MEST
MJD
MNDA
MYL1
NCAM2
OSR2
PDCD4
PIR51
RFC5
RFPL1S
RORB
SERPINB10
SERPINB1
SERPINB2
SFRP1
SIRPB1
SPP1
STAM2
TIAF1
TSN
VELI1
Immune response
AF070578[a]
AL078636
ATRN
CALCRL
CCR2
CCR3
CD1D
CDBA
CDC2
CEACAM6
CNR1
CXCR4
DEFA4
FCGR2A
FER1L3
FSHR
GFR
GLRB
IL13RA1
IL15
IL18
IL8RB
ITK
KCNA4
KCNAB1
LILRA2
MAPK9
MS4A3
OAS1
P2RY2
PAK1
PAK2
PAK7
PRKACB
PRKCQ
PTPN22
PTPRJ
RET
RPS6KA3
RYR3
SCYA23
SLC15A1
STK4
TNFSF10
TRG
TXK
ZW10
DNA and protein binding
ABCC6
AS3
CRHBP
DLGAP1
DMXL1
FNBP3
GTF2I
HYPE
ID1
LYL1
RGS1
RPS26
TAF6L
TF
TOP2B
ZNF261
Vesicles
PACSIN2
RAB5A
SNAP23
STX16
Oncogene
CAV1
CHEK1
CUL1
LGI1
MYB
MYB
RB1
FOSB[a]
RAB27[a]
Transporter
NUP155
RAB3-GAP150
Transcription
ADAM10
ADK
AF041259
ALDH5A1
ALDH6A1
ART1
ATP6V1A1
B4GALT5
BCKDHB
BDH
BS69
CDKN1B
CDS2
CPM
CTSG
D50419
DHFR
DNASEIL1
EIF2S3
ELL2
EPHX1
FBP1
FECH
GATA2
GLUL
GNS
GSR
GSTA4
GTF2B
GYG2
HAL
HIVEP1
IRF7
KR18
KYNU
LILRB1
LOC51172
MCFP
ME1
MED6
MME
MPI
MPO
MPST
MXI1
MYC
NAALAD2
NDUFB6
NEDD4
NFATC3
NFE2
PDE4D
PDE7A
PDGFRL
PGGT1B
PLU-1
PPP1R8
PPP2R1B
PRP17
PRPS1
RAD51
RBM12
RNAH
RNGTT
RPC32
RRM2
SLC26A2
SMAP
SMARCA2
SMARCA3
SNAPC3
SS18
STAT1
TFAP4
TFDP1
TRIP4
TRIP8
U31248
U37251
U95044
UBE2D1
UGT2B15
XDH
Miscellaneous
AF007155
AL022398
BCL11A
C18orf1
CCNG1
CG012
CHN2
DNAJB12
DNC12
DO
DPYSL4
DRD1
F8
FACVL1
HG2259-HT2348
HG2510-HT2606
HG3523-HT4899
HG4679-HT5104
HIRIP3
HIS1
HRY[a]
HS2ST1
ITM2B
KIF2
KIF5B
LOC51097
LRMP
MDM4
NCALD
NCK1
NCOA2
NR4A2[a]
OMD
OSBPL1A
P311
PCTAIRE2BP
PLOD2
RASGRP2
RPE
SIX3
SKD1
SNX7
STAR
STHM
TM75F2
TXNIP
Y43374
U43604
U90916
W28319
W87466
X55989
ESTs
AA143021
AF052146
AL050151
AW043812
CG018
DKFZp566D133
DKFZP586A0522
DKFZP586G011
GASC1
HSU84971
KIAA0096
KIAA0193
KIAA0241
KIAA0390
KIAA0493
KIAA0628
KIAA0711
KIAA0712
KIAA0746
KIAA0752
KIAA0828
KIAA0869
KIAA0918
KIAA0924
KIAA0930
KIAA1128
KIAA1405
L39064
LOC63923
U79277
W28589
W72239

[a] Transcription of the genes was induced and suppressed at different time points.

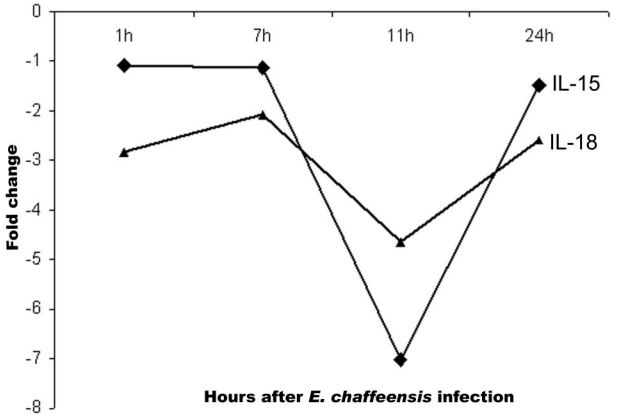

FIG. 3. Repression of transcription of IL-15 and IL-18 genes by *E. chaffeensis*.

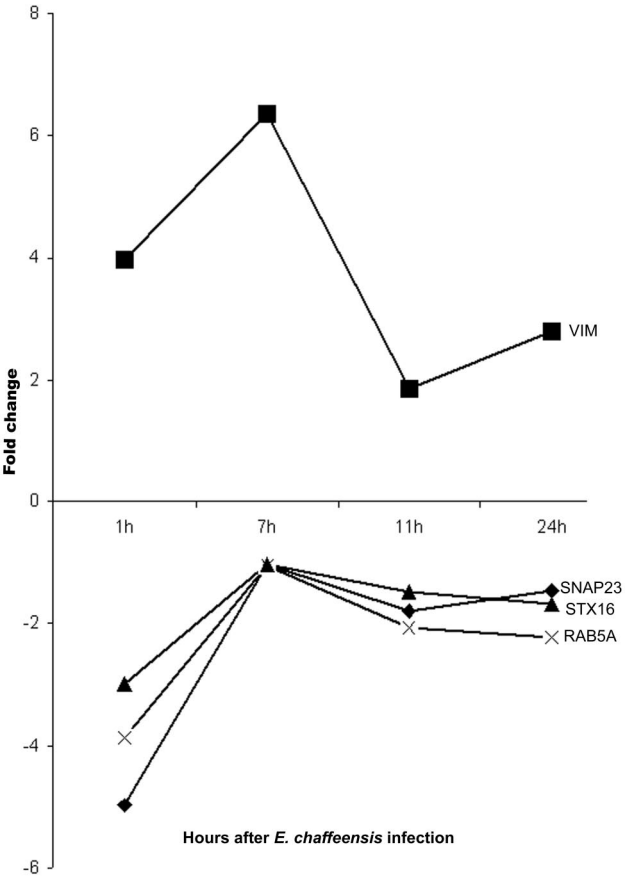

FIG. 4. Regulation of gene transcription of proteins involved in vesicle docking by *E. chaffeensis*.

tosol of the host cell by a host membrane. Considering the nature and scope of these differentially transcribed genes, the interaction between *E. chaffeensis* and the host cell is far more complex than simply fulfilling the metabolic needs of *E. chaffeensis*. *E. chaffeensis* infection results in profound changes in the transcription of host cell genes encoding proteins involved in biosynthesis and metabolism, ion channel transport, regulation of cell differentiation, signal transduction and transcription, inflammation, and membrane trafficking. From the point of view of pathogenesis, the most important changes in the host cell caused by *E. chaffeensis* infection are downregulation of the innate immune system and a differentially regulated cell cycle.

The most striking feature of *E. chaffeensis* infection is repression of host cell cytokines that modulate innate and adaptive immunity to intracellular bacteria. *E. chaffeensis* avoids stimulation of IL-12 production and represses IL-15 and IL-18 production. These cytokines play fundamental roles in stimulating NK cells and T helper 1 cells to produce gamma interferon (IFN-γ), which then activates macrophages to kill phagocytosed bacteria. IL-12 and IL-15 also activate NK cells and cytotoxic T lymphocytes to kill cells infected with intracellular bacteria. Thus, repression of IL-12, IL-15, and IL-18 may help *E. chaffeensis* to evade host innate and adaptive immunity. Another intracellular bacterium, *Mycobacterium tuberculosis* (26), and the intracellular protozoan and fungus *Leishmania major* (9) and *Histoplama capsulatum* (23) inhibit IL-12 production. Thus, intracellular pathogens may have convergently evolved the ability to survive inside the macrophage by repressing IL-12 production.

Apoptosis is an innate mechanism of host defense used to prevent proliferation of internalized bacteria (31). Intracellular bacteria usually grow very slowly and require several days of intracellular replication. Thus, intracellular bacteria such as *M. tuberculosis* (5, 30), *Chlamydia trachomatis* (18), *Rickettsia rickettsii* (10), and *Anaplasma phagocytophilum* (37) have all evolved different mechanisms to inhibit host cell apoptosis during the early stages of infection to gain time for growth within host cells. *E. chaffeensis* induces the production of apoptosis inhibitors such as NF-κB, BCL2A1, BIRC3, IER3, and MCL1. In the early stage of infection (7 h), *E. chaffeensis* represses the BCL2 antagonists BIK and BNIP3L, which induce apoptosis by inactivating BCL2 proteins (8). The expression of BCL2 proteins and their antagonists returns to normal levels gradually in the late stages of infection. However, HCK is induced during the late stages of infection. The HCK SH3 domain mediates signaling at the plasma membrane, triggering a pathway leading to caspase-3-dependent cytochrome *c* release and apoptosis (29). NF-κB stimulates cell proliferation by activating cellular transcription. *R. rickettsii* blocks host cell apoptosis through activation of the NF-κB prosurvival signaling pathway (10). Prosurvival members of the BCL2 family (BCL2, BCL2A1, and MCL1) prevent apoptosis by maintaining the integrity of the mitochondrial membrane and thus preventing the release of cytochrome *c*, which binds to apoptotic protease-activating factor 1, resulting in activation of the apoptosis pathway (19, 31, 40). *C. trachomatis* inhibits host cell apoptosis by blocking the BCL2 pathway. It will be interesting to investigate whether *E. chaffeensis* inhibits apoptosis during the early stage of infection by regulating the mitochondrial release of cytochrome *c*, since our data suggest that *E. chaffeensis* blocks the BCL2 pathway.

E. chaffeensis survival within the macrophage depends on its ability to inhibit phagosome-lysosome fusion. After ingestion by a macrophage, *E. chaffeensis* lives in a vacuole containing early endosomal markers, such as EEA1, but not lysosomal markers, such as LAMP1 (25). Thus, *E. chaffeensis* lives in an

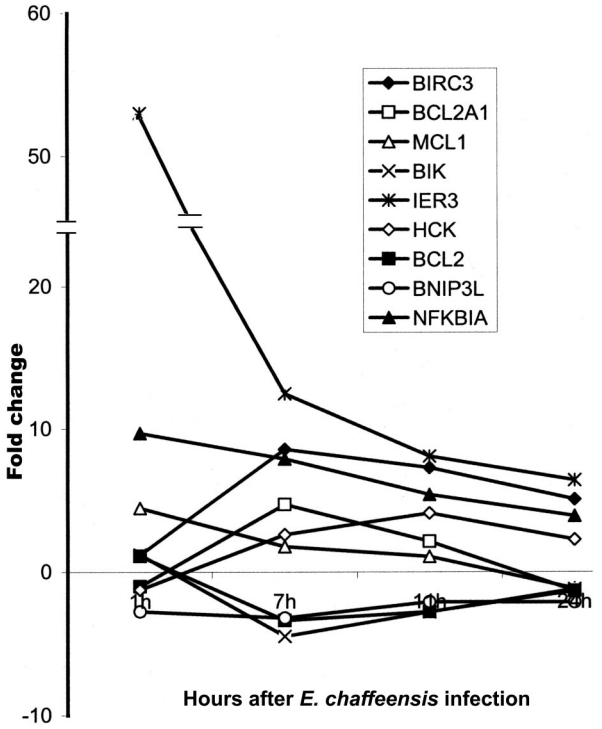

FIG. 5. Differential regulation of gene transcription of apoptosis inhibitors and inducers by *E. chaffeensis*.

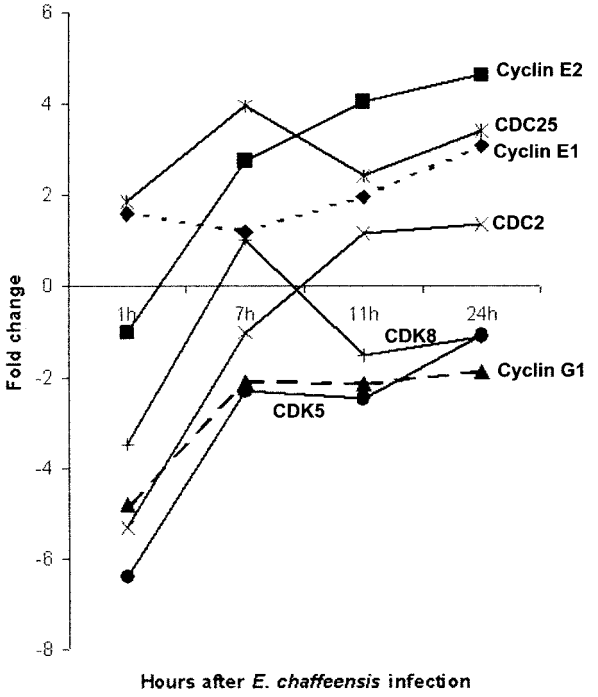

FIG. 6. Differential regulation of gene transcription of proteins involved in the cell cycle by *E. chaffeensis*.

early endosome and inhibits the maturation of the endosome to evade destruction by lysosomal enzymes. The mechanism that *E. chaffeensis* employs to inhibit the maturation of the endosome is not understood. A current model of vesicle fusion is explained by the SNARE hypothesis. According to the SNARE hypothesis, docking and fusion of vesicles with the plasma membrane are mediated by the specific interaction of vesicle proteins (v-SNARE and SNAR receptor) with the target plasma membrane protein (t-SNARE) (34). Among the proteins implicated are syntaxins, which have at least 16 members, synaptosome-associated proteins (SNAPs), of which the two best known are SNAP25 and SNAP23, and other proteins. These proteins form a complex that juxtaposes the two membranes to be fused. This interaction is regulated by Rab5, a small GTPase of the Rab family. Depletion of Rab5 inhibits the fusion of the phagosome containing *Listeria monocytogenes* with lysosomes (2). SNAP23 has been demonstrated to interact with different syntaxins in different types of cells (22, 24). Our data show that *E. chaffeensis* represses the production of Rab5, SNAP23, and STX16 (syntaxin 16) at all times during infection, most dramatically during the first hour of infection. *E. chaffeensis* induces the production of vimentin, a reservoir for SNAP23 (17). Thus, *E. chaffeensis* may inhibit phagosome-lysosome fusion by regulating the concentration of Rab5 and SNAPs in the macrophage.

Protein kinases are essential elements of signal transduction pathways that control fundamental cellular processes, including growth, differentiation, and cytoskeletal function. Protein kinases are activated by phosphorylation of tyrosine, serine, or threonine residues and are inactivated by dephosphorylation by protein phosphatases. *E. chaffeensis* infection regulated 55 protein kinase and kinase-related genes of the host cells.

E. chaffeensis infection downregulated protein kinases involving cell mobility and cytoskeletal changes, such as ITK, TXK, and PAKs. ITK and TXK were inhibited by *E. chaffeensis* at all times during infection. ITK and TXK are nonreceptor tyrosine kinases of the Tec family. ITK regulates TCR/CD3-induced actin cytoskeletal events (20). The expression of ITK was thought to be restricted to T lymphocytes, NK cells, and mast cells (36). TXK binds to the IFN-γ gene promoter region that regulates IFN-γ gene transcription (32). PAK1, PAK2, and PAK7 were downregulated during the first hour of infection. PAKs (p21-activated kinases) serve as important regulators of cytoskeletal dynamics and cell mobility, transcription through mitogen-activated protein (MAP) kinase cascades, death and survival signaling, and cell cycle progression (7).

E. chaffeensis inhibits the JAK-STAT pathway. JAK1 and STAT1 were repressed at the early stage (1 h) of *E. chaffeensis* infection. The JAK-STAT pathway has a fundamental role in cytokine signaling. JAKs bind specifically to intracellular domains of cytokine receptor signaling chains and phosphorylate themselves and tyrosine residues on the receptor, creating docking sites for the SH2 domains of STATs. The receptor-bound STAT is then phosphorylated at a tyrosine residue. Phosphorylation of STAT leads to STAT homo- and heterodimer formation dependent on the intermolecular SH2-phosphotyrosine interaction. STAT dimers are rapidly transported from the cytoplasm to the nucleus and are involved in DNA binding. The ligands for receptors which bind JAKs include IFN-α, -β, and -γ, IL-2 to -7, -10 to -13, and -15, and erythropoietin, growth hormone, prolactin, thrombopoietin,

and other polypeptides (1, 11). Therefore, *E. chaffeensis* may inhibit activation of macrophages by interferons and interleukins by downregulating the JAK-STAT pathway.

E. chaffeensis inhibits cyclin and CDC2-related protein kinases (CDKs). The cell cycles of eukaryotes are controlled by multiple cyclins and multiple CDKs. *E. chaffeensis* downregulates CDC2, CDK5, CDK8, and cyclin G1 during the early stage of infection. In *Saccharomyces cerevisiae*, passage through START is controlled by CDC2 in association with cyclin G1. Thus, *E. chaffeensis* may arrest the host cell in the G_1 stage during the early stages of infection. The early-stage inhibition of host cell growth may help *E. chaffeensis* to establish itself inside the cell during the initial stages of infection. In the late stages of infection, however, *E. chaffeensis* upregulates cell proliferation to prevent the cell from dying due to progressive infection. This hypothesis is supported by our data that *E. chaffeensis* upregulates cyclin E and CDC25 in the late stages of infection. Cyclin E is expressed later in the G_1 phase of the cell cycle and plays a role in the G_1-to-S phase transition and initiation of DNA synthesis.

E. chaffeensis downregulates JNK2 (MAPK9) during the early stage of infection and upregulates DUSP8 and -14 (dual-specificity phosphatase), which dephosphorylate and inactivate JNK2. JNK2 is a member of the MAP kinases. JNK2 phosphorylates the DNA binding protein Jun and increases its transcriptional activity. Jun is a component of the AP-1 transcription factor, which activates transcription of a number of genes in response to environmental stress, radiation, and growth factors (21). JNKs are important in controlling apoptosis. The absence of JNK causes a defect in the mitochondrial death signaling pathway by inhibiting cytochrome *c* release (33). *E. chaffeensis* may inhibit cell transcriptional activity during the early stage of infection and/or inhibit apoptosis through downregulation of MAP kinase pathways, thus impairing host cell defenses and maintaining a prolonged growth opportunity for ehrlichiae.

Other protein kinases are downregulated in the first hour of infection by *E. chaffeensis*, including ADK, CNK, EPHA2, EPHB2, and STK4. ADK is involved in ribonucleoside monophosphate biosynthesis. CNK is required for Raf activation (4). The EphA2 receptor tyrosine kinase critically regulates tumor cell growth, migration, and invasiveness (28). A primary function of EphB2, a member of the most populous family of receptor tyrosine kinases, is to inactivate the Ras-MAP kinase pathway in a fashion that contributes to cytoskeletal reorganization and adhesion responses in neuronal growth cones (15).

A previous study compared the transcriptional profiles of macrophages infected with mycobacteria, gram-negative bacteria (*E. coli*, *Salmonella enterica* serovar Typhi, and *S. enterica* serovar Typhimurium), and gram-positive bacteria (*Staphylococcus aureus* and *L. monocytogenes*) and identified shared transcriptional responses among the bacteria, consisting of 132 induced genes and 59 repressed genes (9). Despite the fact that a similar number of genes changed transcriptional levels in macrophages infected with *E. chaffeensis* and macrophages infected with other bacteria, the transcriptional profile of *E. chaffeensis*-infected macrophages differed remarkably from that of macrophages infected with other bacteria, as mentioned above. In comparing the shared transcriptional response of macrophages infected with other bacteria and that of macrophages infected with *E. chaffeensis*, we have identified only a few genes that are commonly induced by *E. chaffeensis* and the other bacteria and found no shared repressed genes. The commonly induced genes include those involved in the innate immune response and the stress response (IL-8, IL-7R, and SOD2), transcription (JunB, NFKBIA, and NFKBIE), and cell adhesion (ICAM1). It is very interesting that another intracellular bacterium, *Brucella abortus*, also inhibits macrophage transcription of various genes involved in apoptosis, cell cycling, and intracellular vesicular trafficking (16), although *E. chaffeensis* and *B. abortus* inhibited different genes involved in these processes.

ACKNOWLEDGMENTS

We are grateful to S. Wesley Long and David H. Walker for discussions of the manuscript.

This research was supported by a grant from the National Institute of Allergy and Infectious Diseases (AI45871).

REFERENCES

1. **Aaronson, D. S., and C. M. Horvath.** 2002. A road map for those who know JAK-STAT. Science **296:**1653–1655.
2. **Alvarez-Dominguez, C., A. M. Barbieri, W. Beron, A. Wandinger-Ness, and P. D. Stahl.** 1996. Phagocytosed live *Listeria monocytogenes* influences Rab5-regulated in vitro phagosome-endosome fusion. J. Biol. Chem. **271:**13834–13843.
3. **Anderson, B. E., K. G. Sims, J. G. Olson, J. E. Childs, J. F. Piesman, C. M. Happ, G. O. Maupin, and B. J. Johnson.** 1993. *Amblyomma americanum*: a potential vector of human ehrlichiosis. Am. J. Trop. Med. Hyg. **49:**239–244.
4. **Anselmo, A. N., R. Bumeister, J. M. Thomas, and M. A. White.** 2002. Critical contribution of linker proteins to Raf kinase activation. J. Biol. Chem. **277:**5940–5943.
5. **Balcewicz-Sablinska, M. K., J. Keane, H. Kornfeld, and H. G. Remold.** 1998. Pathogenic Mycobacterium tuberculosis evades apoptosis of host macrophages by release of TNF-R2, resulting in inactivation of TNF-alpha. J. Immunol. **161:**2636–2641.
6. **Barnewall, R. E., Y. Rikihisa, and E. H. Lee.** 1997. *Ehrlichia chaffeensis* inclusions are early endosomes which selectively accumulate transferrin receptor. Infect. Immun. **65:**1455–1461.
7. **Bokoch, G. M.** 2003. Biology of the p21-activated kinases. Annu. Rev. Biochem. **72:**743–781.
8. **Boyd, J. M., G. J. Gallo, B. Elangovan, A. B. Houghton, S. Malstrom, B. J. Avery, R. G. Ebb, T. Subramanian, T. Chittenden, R. J. Lutz, et al.** 1995. Bik, a novel death-inducing protein shares a distinct sequence motif with Bcl-2 family proteins and interacts with viral and cellular survival-promoting proteins. Oncogene **11:**1921–1928.
9. **Carrera, L., R. T. Gazzinelli, R. Badolato, S. Hieny, W. Muller, R. Kuhn, and D. L. Sacks.** 1996. *Leishmania promastigotes* selectively inhibits interleukin 12 induction in bone marrow-derived macrophages from susceptible and resistant mice. J. Exp. Med. **183:**515–526.
10. **Clifton, D. R., R. A. Goss, S. K. Sahni, D. van Antwerp, R. B. Baggs, V. J. Marder, D. J. Silverman, and L. A. Sporn.** 1998. NF-kappa B-dependent inhibition of apoptosis is essential for host cell survival during *Rickettsia rickettsii* infection. Proc. Natl. Acad. Sci. USA **95:**4646–4651.
11. **Darnell, J. E., Jr.** 1997. STATs and gene regulation. Science **277:**1630–1635.
12. **Davidson, W. R., J. M. Lockhart, D. E. Stallknecht, E. W. Howerth, J. E. Dawson, and Y. Rechav.** 2001. Persistent *Ehrlichia chaffeensis* infection in white-tailed deer. J. Wildl. Dis. **37:**538–546.
13. **Dawson, J. E., B. E. Anderson, D. B. Fishbein, J. L. Sanchez, C. S. Goldsmith, K. H. Wilson, and C. W. Duntley.** 1991. Isolation and characterization of an *Ehrlichia* sp. from a patient diagnosed with human ehrlichiosis. J. Clin. Microbiol. **29:**2741–2745.
14. **Dawson, J. E., and S. A. Ewing.** 1992. Susceptibility of dogs to infection with *Ehrlichia chaffeensis*, causative agent of human ehrlichiosis. Am. J. Vet. Res. **53:**1322–1327.
15. **Elowe, S., S. J. Holland, S. Kulkarni, and T. Pawson.** 2001. Downregulation of the Ras–mitogen-activated protein kinase pathway by the EphB2 receptor tyrosine kinase is required for ephrin-induced neurite retraction. Mol. Cell. Biol. **21:**7429–7441.
16. **Eskra, L., A. Mathison, and G. Splitter.** 2003. Microarray analysis of mRNA levels from RAW264.7 macrophages infected with *Brucella abortus*. Infect. Immun. **71:**1125–1133.
17. **Faigle, W., E. Colucci-Guyon, D. Louvard, S. Amigorena, and T. Galli.** 2000. Vimentin filaments in fibroblasts are a reservoir for SNAP23, a component of the membrane fusion machinery. Mol. Biol. Cell **11:**3485–3494.

18. **Fan, T., H. Lu, H. Hu, L. Shi, G. A. McClarty, D. M. Nance, A. H. Greenberg, and G. Zhong.** 1998. Inhibition of apoptosis in *Chlamydia*-infected cells: blockade of mitochondrial cytochrome c release and caspase activation. J. Exp. Med. **187:**487–496.
19. **Gao, L. Y., and Y. A. Kwaik.** 2000. The modulation of host cell apoptosis by intracellular bacterial pathogens. Trends Microbiol. **8:**306–313.
20. **Grasis, J. A., C. D. Browne, and C. D. Tsoukas.** 2003. Inducible T cell tyrosine kinase regulates actin-dependent cytoskeletal events induced by the T cell antigen receptor. J. Immunol. **170:**3971–3976.
21. **Johnson, G. L., and R. Lapadat.** 2002. Mitogen-activated protein kinase pathways mediated by ERK, JNK, and p38 protein kinases. Science **298:** 1911–1912.
22. **Lazo, P. A., M. Nadal, M. Ferrer, E. Area, J. Hernandez-Torres, S. M. Nabokina, F. Mollinedo, and X. Estivill.** 2001. Genomic organization, chromosomal localization, alternative splicing, and isoforms of the human synaptosome-associated protein-23 gene implicated in vesicle-membrane fusion processes. Hum. Genet. **108:**211–215.
23. **Marth, T., and B. L. Kelsall.** 1997. Regulation of interleukin-12 by complement receptor 3 signaling. J. Exp. Med. **185:**1987–1995.
24. **Martin-Martin, B., S. M. Nabokina, P. A. Lazo, and F. Mollinedo.** 1999. Co-expression of several human syntaxin genes in neutrophils and differentiating HL-60 cells: variant isoforms and detection of syntaxin 1. J. Leukoc. Biol. **65:**397–406.
25. **Mott, J., R. E. Barnewall, and Y. Rikihisa.** 1999. Human granulocytic ehrlichiosis agent and *Ehrlichia chaffeensis* reside in different cytoplasmic compartments in HL-60 cells. Infect. Immun. **67:**1368–1378.
26. **Nau, G. J., J. F. Richmond, A. Schlesinger, E. G. Jennings, E. S. Lander, and R. A. Young.** 2002. Human macrophage activation programs induced by bacterial pathogens. Proc. Natl. Acad. Sci. USA **99:**1503–1508.
27. **Paddock, C. D., and J. E. Childs.** 2003. *Ehrlichia chaffeensis*: a prototypical emerging pathogen. Clin. Microbiol. Rev. **16:**37–64.
28. **Pratt, R. L., and M. S. Kinch.** 2002. Activation of the EphA2 tyrosine kinase stimulates the MAP/ERK kinase signaling cascade. Oncogene **21:**7690–7699.
29. **Radha, V., C. Sudhakar, P. Ray, and G. Swarup.** 2002. Induction of cytochrome c release and apoptosis by Hck-SH3 domain-mediated signalling requires caspase-3. Apoptosis **7:**195–207.
30. **Rojas, M., M. Olivier, P. Gros, L. F. Barrera, and L. F. Garcia.** 1999. TNF-alpha and IL-10 modulate the induction of apoptosis by virulent Mycobacterium tuberculosis in murine macrophages. J. Immunol. **162:**6122–6131.
31. **Sly, L. M., S. M. Hingley-Wilson, N. E. Reiner, and W. R. McMaster.** 2003. Survival of Mycobacterium tuberculosis in host macrophages involves resistance to apoptosis dependent upon induction of antiapoptotic Bcl-2 family member Mcl-1. J. Immunol. **170:**430–437.
32. **Takeba, Y., H. Nagafuchi, M. Takeno, J. Kashiwakura, and N. Suzuki.** 2002. Txk, a member of nonreceptor tyrosine kinase of Tec family, acts as a Th1 cell-specific transcription factor and regulates IFN-gamma gene transcription. J. Immunol. **168:**2365–2370.
33. **Tournier, C., P. Hess, D. D. Yang, J. Xu, T. K. Turner, A. Nimnual, D. Bar-Sagi, S. N. Jones, R. A. Flavell, and R. J. Davis.** 2000. Requirement of JNK for stress-induced activation of the cytochrome c-mediated death pathway. Science **288:**870–874.
34. **Weber, T., B. V. Zemelman, J. A. McNew, B. Westermann, M. Gmachl, F. Parlati, T. H. Sollner, and J. E. Rothman.** 1998. SNAREpins: minimal machinery for membrane fusion. Cell **92:**759–772.
35. **Weiss, E., J. C. Williams, G. A. Dasch, and Y. H. Kang.** 1989. Energy metabolism of monocytic Ehrlichia. Proc. Natl. Acad. Sci. USA **86:**1674–1678.
36. **Yang, W. C., Y. Collette, J. A. Nunes, and D. Olive.** 2000. Tec kinases: a family with multiple roles in immunity. Immunity **12:**373–382.
37. **Yoshiie, K., H. Y. Kim, J. Mott, and Y. Rikihisa.** 2000. Intracellular infection by the human granulocytic ehrlichiosis agent inhibits human neutrophil apoptosis. Infect. Immun. **68:**1125–1133.
38. **Yu, X. J., P. A. Crocquet-Valdes, L. C. Cullman, V. L. Popov, and D. H. Walker.** 1999. Comparison of *Ehrlichia chaffeensis* recombinant proteins for serologic diagnosis of human monocytotropic ehrlichiosis. J. Clin. Microbiol. **37:**2568–2575.
39. **Yu, X. J., J. W. McBride, C. M. Diaz, and D. H. Walker.** 2000. Molecular cloning and characterization of the 120-kilodalton protein gene of *Ehrlichia canis* and application of the recombinant 120-kilodalton protein for serodiagnosis of canine ehrlichiosis. J. Clin. Microbiol. **38:**369–374.
40. **Zhou, P., L. Qian, K. M. Kozopas, and R. W. Craig.** 1997. Mcl-1, a Bcl-2 family member, delays the death of hematopoietic cells under a variety of apoptosis-inducing conditions. Blood **89:**630–643.

Editor: W. A. Petri, Jr.