Transcriptome Analysis of NF- κ B- and Phosphatidylinositol 3-Kinase-Regulated Genes in Human Cytomegalovirus-Infected Monocytes^{∇†}

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Human cytomegalovirus induces a proinflammatory monocyte following infection, and we have evidence that NF- κ B and phosphatidylinositol 3-kinase [PI(3)K] are key mediators in this early activation. To begin to address how these signaling pathways are responsible for the rapid activation of infected monocytes, we examined the role that these pathways played in the transcriptome of infected monocytes. Global transcriptional profiling using cDNA microarrays revealed that a significant number of genes, including inflammatory genes, were regulated in an NF- κ B- and/or PI(3)K-dependent manner, identifying the NF- κ B and PI(3)K pathways as key cellular control points in the conversion of monocytes to an activated proinflammatory state following HCMV infection.

Human cytomegalovirus (HCMV) viremia is cell associated, and it is widely believed that hematogenous spread occurs via the action of peripheral blood leukocytes, especially monocytes (8, 11, 12). We previously provided evidence suggesting that HCMV utilizes monocytes as a vehicle for spread, infiltration into, and persistence in host tissue by promoting the acquisition of a proinflammatory phenotype (13–15). Activated monocytes exhibit increased cell motility, firm adhesion to endothelial cells, transendothelial migration, and differentiation from short-lived monocytes (nonpermissive for viral replication) into long-lived macrophages (permissive for viral replication) (1, 13–15).

We found that NF- κ B and phosphatidylinositol 3-kinase [PI(3)K] activities are rapidly induced in monocytes following infection (14, 17), suggesting that the NF- κ B and PI(3)K pathways could serve as the molecular regulators for the rapid functional changes we observed in monocytes following infection (13–15, 17). Indeed, earlier studies examining the role that NF- κ B and PI(3)K activity played in regulating monocyte function after infection showed that inhibition of NF- κ B and PI(3)K activity blocked a number of HCMV-induced proinflammatory characteristics, including cell motility, adhesion to endothelial cells, transendothelial migration, and monocyte-to-macrophage differentiation (14, 15). To begin to globally define how NF- κ B and PI(3)K are involved in the HCMV-induced changes in monocyte function, we performed a transcriptome analysis in the presence of inhibitors to NF- κ B and PI(3)K signaling pathways. Specifically, a cDNA microar-

ray containing 12,626 unique probe sets was utilized to assess the modulation of the monocyte transcriptome at 4 h postinfection (hpi) in the presence of the pharmacological agents Bay11-7082 (an NF- κ B inhibitor) (2) and LY294002 [a PI(3)K inhibitor] (14).

Affymetrix gene array and analysis. Isolated monocytes were pretreated nonadherently at 37°C for 45 min with 5 μ M of the NF- κ B inhibitor Bay11-7082 (Calbiochem, San Diego, CA), 50 μ M of the PI(3)K inhibitor LY294002 (Promega, Madison, WI) (2, 14), or dimethyl sulfoxide as the solvent control prior to infection. Dose-response studies using Bay11-7082 and LY294002 showed no toxicity to monocytes through 72 h at these concentrations, while still inhibiting cell motility and transendothelial migration (data not shown). Cells were then HCMV infected and incubated nonadherently at 37°C. At 4 hpi, the cells were pelleted, and the total RNA was isolated with the RNA STAT-60 isolation kit (Tel-Test, Friendswood, TX) according to the manufacturer's protocol.

Affymetrix human genome U95Av2 arrays (Affymetrix, Santa Clara, CA), which contain 12,626 characterized sequences (relating to 11,266 genes), were used to examine the cellular gene changes in primary human monocytes from multiple human donors. The total RNA was harvested as described above from dimethyl sulfoxide-pretreated, HCMV-infected; LY294002-pretreated, HCMV-infected; and, Bay11-7082-pretreated, HCMV-infected monocytes. RNA was reverse transcribed into cDNA and then transcribed in vitro to biotin-labeled cDNA. cDNA from each sample was fragmented and hybridized to GeneChip expression arrays, and the data were analyzed using Affymetrix Microarray Suite version 5.0. Data Mining Tool version 3.0 was used to compile data from each of the replicates, and one-way analysis of variance tests were performed to calculate *P* values. Spotfire DecisionSite 8.1.1 (TIBCO, Somerville, MA) was used to group genes by ontology, generate scatter plots, and calculate the coefficient of determination (R^2).

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FIG. 1. Linear regression analyses of Bay11-7082 (Bay11)-pretreated and/or LY294002 (LY)-pretreated HCMV-infected replicates. Statistically significant genes from Bay11-7082-pretreated, HCMV-infected samples; LY294002-pretreated, HCMV-infected samples; and Bay11-7082- and LY294002-pretreated, HCMV-infected samples were plotted on the basis of signal. Squares represent individual genes, and the “line of best fit” on each graph was used to calculate the coefficient of determination (R^2). The scatter plots were generated with Spotfire DecisionSite 8.1.1 software.

Because we previously identified NF- κ B and PI(3)K as key players in the regulation of the HCMV-induced inflammatory phenotype (13–15, 17), we focused our transcriptome analysis of HCMV-infected monocytes on the NF- κ B- and PI(3)K-regulated genes. Cellular genes dependent upon NF- κ B or PI(3)K activity were determined by comparing the genes in HCMV-infected replicates to those that were downregulated an average of 1.5-fold or more in Bay11-7082-pretreated or LY294002-pretreated, HCMV-infected samples. Experiments were performed in triplicate, and the data from each replicate were compiled for statistical analyses with the following criterion: an average decrease of 1.5-fold or more in gene expression following pretreatment with Bay11-7082 or LY294002 prior to infection. Next, one-way analysis of variance tests were performed on the HCMV-infected versus Bay11-7082-pre-

treated or LY294002-pretreated replicates and P values were calculated for genes that were downregulated. A P value of ≤ 0.05 was used as the criterion for statistically significant genes among replicates. Moreover, a present call (a detection algorithm using probe pair intensities to generate a detection P value and the Affymetrix defined threshold was used to assign a present, marginal, or absent call) in the HCMV-infected replicates of at least two of three donors was required for classification of dependence on NF- κ B and PI(3)K activity; thus, genes that may otherwise have been eliminated due to anomalous expression by a single donor were accepted. Genes dependent on both NF- κ B and PI(3)K activity were determined by comparing NF- κ B-dependent and PI(3)K-dependent genes in Microsoft Access (Microsoft Office, XP Professional).

TABLE 1. Select statistically significant mRNA levels that decrease ≥ 1.5 -fold after treatment with Bay11-7082 or LY294002 prior to HCMV infection^a

Category and full gene product name	Gene product designation	Probe set ID	Fold change in mRNA level by treatment with:	
			Bay11-7082 ^b	LY294002 ^b
Antiapoptotic factors				
Baculoviral IAP repeat-containing 2	BIRC2	36578_at	-1.8	
BCL2-related protein A1	BCL2A1	2002_s_at	-1.6	
Beclin 1 (coiled-coil, myosin-like BCL2 interacting protein)	BECN1	39378_at	-1.9	
CASP8 and FADD-like apoptosis regulator	CFLAR	1867_at	-2.8	
Chemokine (C-C motif) ligand 2	CCL2	874_at	-3.5	-55.2
Heat shock 70-kDa protein 9B (mortalin 2)	HSPA9B	41510_s_at		-1.9
Immediate early response 3	IER3	1237_at	-2.0	
Interleukin 10	IL-10	1548_s_at	-8.4	
Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 (p105)	NFKB1	1377_at	-2.8	
Secreted phosphoprotein 1 (osteopontin, bone sialoprotein I, early T-lymphocyte activation 1)	SPP1	2092_s_at	-4.7	-18.5
Serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 9	SERPINB9	2092_s_at	-4.8	
Suppressor of cytokine signaling 3	SOCS3	34438_at	-2.5	
Tumor necrosis factor (tumor necrosis factor superfamily, member 2)	TNF	40968_at	-4.1	
Tumor necrosis factor, alpha-induced protein 3	TNFAIP3	1852_at	-4.0	
Tumor necrosis factor, alpha-induced protein 8	TNFAIP8	595_at	-4.4	
Tumor rejection antigen (gp96) 1	TRA1	33243_at		-1.67
Cell adhesion				
ADAM-like, decysin 1	ADAMDEC1	34974_at	-3.1	-9.3
Angiogenic factor VG5Q	VG5Q	35067_at	-1.7	
Cadherin 1, type 1, E-cadherin (epithelial)	CDH1	977_s_at	-20.7	
Catenin (cadherin-associated protein), delta 1	CTNND1	40444_s_at	-2.0	
CD44 antigen (homing function and Indian blood group system)	CD44	31472_s_at	-1.5	
CD47 antigen (Rh-related antigen, integrin-associated signal transducer)	CD47	37890_at	-2.6	
CD9 antigen (p24)	CD9	39389_at		-2.5
Chemokine (C-C motif) ligand 2	CCL2	34375_at	-3.5	-55.2
Chemokine (C-C motif) ligand 4	CCL4	36674_at	-7.1	
Complement component 1, q subcomponent, receptor 1	C1QR1	35036_at		-4.6
Endoglin (Osler-Rendu-Weber syndrome 1)	ENG	32562_at	-4.4	
Heat shock 60-kDa protein 1 (chaperonin)	HSPD1	37720_at		-2.0
Integrin, alpha 1	ITGA1	37484_at	-2.3	-9.6
Integrin, alpha 6	ITGA6	41266_at	-2.5	2.5
Integrin, beta 8	ITGB8	889_at	-7.9	-5.5
Intercellular adhesion molecule 1 (CD54), human rhinovirus receptor	ICAM1	32640_at	-4.7	
Laminin, beta 3	LAMB3	36929_at	-2.6	-3.7
Ninjurin 1	NINJ1	41475_at	-2.0	-2.1
Oxidized low-density lipoprotein (lectin-like) receptor 1	OLR1	37233_at	-1.6	
Pleckstrin homology domain containing, family C (with FERM domain) member 1	PLEKHC1	36577_at		-2.7
Polycystic kidney disease 2 (autosomal dominant)	PKD2	38120_at	-2.4	
RAB13, member RAS oncogene family	RAB13	40210_at	-4.9	-2.0
Scavenger receptor class F, member 1	SCARF1	40034_r_at		-3.6
Secreted phosphoprotein 1 (osteopontin, bone sialoprotein I, early T-lymphocyte activation 1)	SPP1	2092_s_at	-4.7	-18.5
Thrombospondin 1	THBS1	115_at	-8.9	-3.1
Tumor necrosis factor (TNF superfamily, member 2)	TNF	1852_at	-4.1	
Tumor necrosis factor, alpha-induced protein 6	TNFAIP6	1372_at	-14.3	
Cell motility				
CD9 antigen (p24)	CD9	39389_at		-2.5
Chemokine (C-C motif) ligand 3	CCL3	36103_at	-3.9	
Chemokine (C-C motif) ligand 4	CCL4	36674_at	-7.1	
Complement component 3a receptor 1	C3AR1	32068_at	-4.2	
Interleukin 10	IL-10	1548_s_at	-8.4	
Lymphocyte-specific protein 1	LSP1	36493_at		-2.4
Myristoylated alanine-rich protein kinase C substrate	MARCKS	32434_at	-1.8	
Phosphatase and tensin homolog (mutated in multiple advanced cancers 1)	PTEN	39552_at	-2.0	
Prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)	PTGS2	1069_at	-5.7	
Rho guanine nucleotide exchange factor (GEF) 11	ARHGEF11	39281_at	-2.1	
Secreted phosphoprotein 1 (osteopontin, bone sialoprotein I, early T-lymphocyte activation 1)	SPP1	2092_s_at	-4.7	-18.5
Thrombospondin 1	THBS1	115_at	-8.9	-3.1

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TABLE 1—Continued

Category and full gene product name	Gene product designation	Probe set ID	Fold change in mRNA level by treatment with:	
			Bay11-7082 ^b	LY294002 ^b
Cytokine and chemokine activity				
ATPase, H+ transporting, lysosomal accessory protein 1	ATP6AP1	35770_at	-2	-1.5
Chemokine (C-C motif) ligand 18 (pulmonary and activation-regulated)	CCL18	32128_at		-3.6
Chemokine (C-C motif) ligand 2	CCL2	874_at	-3.5	-55.2
Chemokine (C-C motif) ligand 20	CCL20	40385_at	-11.6	
Chemokine (C-C motif) ligand 22	CCL22	34041_at	-20.2	
Chemokine (C-C motif) ligand 23	CCL23	36444_s_at		-4.6
Chemokine (C-C motif) ligand 3	CCL3	36103_at	-3.9	
Chemokine (C-C motif) ligand 4	CCL4	36674_at	-7.1	
Chemokine (C-C motif) ligand 7	CCL7	39802_at		-23.5
Chemokine (C-C motif) ligand 8	CCL8	37823_at	-1.9	
Colony-stimulating factor 2 (granulocyte-macrophage)	CSF2	1400_at	-2.9	
Colony-stimulating factor 3 (granulocyte)	CSF3	1334_s_at	-39.0	
Granulin	GRN	41198_at		-1.8
Inhibin, beta A (activin A, activin AB alpha polypeptide)	INHBA	40357_at		-6.9
Interferon, alpha 1	IFNA1	1666_at	-5.7	
Interferon, alpha 10	IFNA10	1022_f_at	-3.0	
Interferon, alpha 13	IFNA13	1540_f_at	-5.9	
Interferon, alpha 2	IFNA2	1791_s_at	-3.4	
Interferon, alpha 4	IFNA4	1580_f_at	-10.7	
Interleukin 1 receptor antagonist	IL1RN	37603_at		-5.2
Interleukin 10	IL-10	1548_s_at	-8.5	
Interleukin 6 (interferon, beta 2)	IL-6	38299_at	-13.6	
Lymphotoxin beta (TNF superfamily, member 3)	LTB	40729_s_at		-1.8
Macrophage migration inhibitory factor (glycosylation-inhibiting factor)	MIF	895_at		-1.7
Oncostatin M	OSM	1579_at		-11.6
Secreted phosphoprotein 1 (osteopontin, bone sialoprotein I, early T-lymphocyte activation 1)	SPP1	2092_s_at	-4.7	-18.5
Tumor necrosis factor (ligand) superfamily, member 10	TNFSF10	1715_at	-5.6	
Tumor necrosis factor (ligand) superfamily, member 8	TNFSF8	33012_at	-3.4	
Tumor necrosis factor (TNF superfamily, member 2)	TNF	1852_at	-4.1	
Differentiation				
Agrin	AGRN	33454_at	-1.9	
Angiogenic factor VG5Q	VG5Q	35067_at	-1.7	
Aryl-hydrocarbon receptor nuclear translocator 2	ARNT2	35352_at		-24.3
CD80 antigen (CD28 antigen ligand 1, B7-1 antigen)	CD80	35015_at	-4.8	
CD9 antigen (p24)	CD9	39389_at		-2.5
Colony-stimulating factor 3 (granulocyte)	CSF3	1334_s_at	-39.0	
Fibroblast growth factor 2 (basic)	FGF2	1828_s_at	-7.9	
fms-related tyrosine kinase 1 (vascular endothelial growth factor/vascular permeability factor receptor)	FLT1	1545_g_at	-6.4	
Histone deacetylase 9	HDAC9	37483_at	-7.8	-18.7
Inhibin, beta A (activin A, activin AB alpha polypeptide)	INHBA	37483_at		-6.9
Interferon, gamma-inducible protein 16	IFI16	40357_at	-2.3	
Interleukin 10	IL-10	1456_s_at	-8.4	
Phosphatidylserine receptor	PTDSR	1548_s_at		-2.0
ras homolog gene family, member H	RHOH	35851_g_at	-2.0	
Secreted phosphoprotein 1 (osteopontin, bone sialoprotein I, early T-lymphocyte activation 1)	SPP1	37416_at	-4.7	-18.5
SH2 domain protein 2A	SH2D2A	34432_at	-2.7	
Tumor necrosis factor, alpha-induced protein 2	TNFAIP2	38631_at	-3.1	
Extracellular matrix				
Agrin	AGRN	33454_at	-1.9	
Laminin, beta 3	LAMB3	36929_at	-2.6	-3.7
Matrix metalloproteinase 14 (membrane-inserted)	MMP14	160020_at	-2.7	
Matrix metalloproteinase 7 (matrilysin, uterine)	MMP7	668_s_at		4.1
Matrix metalloproteinase 9 (gelatinase B, 92-kDa gelatinase, 92-kDa type IV collagenase)	MMP9	31859_at		10.6
Secreted phosphoprotein 1 (osteopontin, bone sialoprotein I, early T-lymphocyte activation 1)	SPP1	2092_s_at	-4.7	18.5
Zona pellucida glycoprotein 3 (sperm receptor)	ZP3	39720_g_at	-1.7	

^a BAY11-7802, pharmacological inhibitor of NF- κ B; LY294002, pharmacological inhibitor of PI(3)K.^b BAY11-7802 and LY294002 were incubated with monocytes for 1 h prior to infection with HCMV.

FIG. 2. HCMV infection modulates the monocyte transcriptome in an NF- κ B- and PI(3)K-dependent manner. NF- κ B- and/or PI(3)K-regulated genes were grouped into functional categories. Ontologies generated using Spotfire DecisionSite software were based on the Gene Ontology Consortium database. Statistically significant genes downregulated 1.5-fold or more in HCMV-infected replicates were compared to Bay11-7082 (Bay11)-pretreated or LY294002 (LY)-pretreated, HCMV-infected replicates. The percentages of genes regulated by the NF- κ B pathway, the PI(3)K pathway, and both pathways are depicted by dark gray, white, and light gray bars, respectively. The specific number of genes changed is represented on each bar. The total number of genes analyzed in each ontology category is shown within parentheses.

Roles of PI(3)K and NF- κ B in the HCMV-mediated changes in the monocyte transcriptome. Scatter plots were generated, and the coefficient of determination was calculated (Fig. 1). When comparisons of Bay11-7082-pretreated to Bay11-7082-pretreated samples or of LY294002-pretreated to LY294002-pretreated HCMV-infected samples were performed, coefficients of determination of 0.88 or greater were obtained, confirming the strong linear relationship of the different samples and the reproducibility of the transcription changes between donors. Conversely, pairwise comparisons of Bay11-7082- and LY294002-pretreated, HCMV-infected samples resulted in a lower coefficient of determination (~ 0.72), indicating that a large number of transcripts were differentially regulated by NF- κ B and PI(3)K in HCMV-infected monocytes. This differential regulation of gene products by NF- κ B and PI(3)K is consistent with our earlier finding showing differential regulation of monocyte adhesion-mediated events by NF- κ B and PI(3)K (15).

Consequently, we identified genes that were regulated in a statistically significant manner by the NF- κ B and PI(3)K signaling pathways by use of the selection criteria described above (Table 1). Table 1 lists select statistically significant genes grouped by ontology that were regulated in an NF- κ B- and/or PI(3)K-dependent manner [see Table S1 in the supplemental material for all NF- κ B-regulated genes and Table S2 in the supplemental material for all PI(3)K regulated genes]. Inhibition of NF- κ B activity in infected monocytes resulted in the downregulation of 501 cellular genes, and inhibition of PI(3)K activity in infected cells resulted in the downregulation of 311 cellular genes [Fig. 2, which shows a representation of the NF- κ B- and PI(3)K-regulated genes in the infected monocyte transcriptome]. Expression of 80 genes was found to be impaired in the absence of either NF- κ B or PI(3)K activity. PI(3)K activity can stimulate the transactivation potential of the NF- κ B p65 subunit, thus providing one explanation for the partial overlap in genes regulated by both NF- κ B and PI(3)K (7). It is also possible that both pathways may feed independently into the induction of a particular gene. Overall, 7.9% of

the total transcripts examined were regulated in an NF- κ B or PI(3)K-dependent manner at 4 hpi. Specifically, 5.1% and 3.5% of the genes detected in the HCMV-infected monocyte transcriptome were regulated by NF- κ B and PI(3)K, respectively [percentages are greater than the total due to the overlap of genes that are regulated by both NF- κ B and PI(3)K activity] (Fig. 2).

NF- κ B and PI(3)K activities can regulate the transcription of a number of antiapoptotic genes. Gene array analysis indicates that 14 antiapoptotic factor mRNAs (9.5% of the total number of antiapoptotic factor transcripts examined) were downregulated in the presence of Bay11-7082 or LY294002, including BCL2A1, c-FLIP, monocyte chemoattractant protein 1, interleukin-10, the p65 subunit of NF- κ B, and tumor necrosis factor alpha (TNF- α). Our results are consistent with previous studies which found the induction of TNF- α and monocyte chemoattractant protein 1 to be mediated by NF- κ B and PI(3)K, respectively (6, 16). Viruses inhibit apoptosis by upregulating caspase 8 and FADD-like apoptosis regulator (c-FLIP) to promote virus survival within cells (3, 5). It is possible that HCMV employs such a mechanism to secure its own survival within monocytes. Furthermore, our data also provide insight into the regulation of c-FLIP. The expression of c-FLIP can be regulated via multiple pathways, such as PI(3)K/Akt or NF- κ B; however, the relative contributions of these signaling pathways to the modulation of c-FLIP remain elusive and might be cell type dependent (9, 10). Our data suggest that c-FLIP induction is mediated solely by NF- κ B in monocytes. Overall, all but two antiapoptotic transcripts were observed to be regulated by NF- κ B, suggesting that the rapid induction of NF- κ B activity is largely responsible for the expression of antiapoptotic genes during the early times of infection.

Inflammatory RNAs involved in cell adhesion (16.7%), cell motility (48%), cytokine/chemokine activity (13%), differentiation (35.4%), and production of the extracellular matrix (4.4%) were rapidly regulated by NF- κ B and/or PI(3)K activity. Reverse transcription-PCR confirmed the inhibition of intercellular adhesion molecule 1, integrin β 8, integrin α 1, interleukin-1 β , and TNF- α mRNA expression by Bay11-7082 treatment and CCL2, integrin β 8, and integrin α 1 mRNA expression by LY294002 treatment (data not shown). In comparison to the 7.9% of the total genes regulated by NF- κ B and/or PI(3)K, the disproportionately high percentage of inflammatory state-associated genes regulated by these two factors (with the exception of extracellular matrix-associated genes) suggest that NF- κ B and PI(3)K are key modulators of the HCMV-induced inflammatory monocyte. Moreover, a detailed examination of the NF- κ B pathway using Ingenuity Pathway Analysis software (Redwood City, CA) indicates that many of the components responsible for positive regulation of NF- κ B activity were upregulated at the transcript level (Fig. 3). In contrast, key components of other cellular signaling pathways, such as the extracellular signal-regulated kinase, p38, and Jun N-terminal protein kinase pathways which lead to the activation of alternative transcription factors, were downregulated at the transcript level (see Fig. S1 in the supplemental material). This apparent selective upregulation of the NF- κ B pathway may ensure that NF- κ B activity remains at high levels during the HCMV-induced monocyte inflammatory state,

FIG. 3. HCMV regulates the expression of multiple components of the NF- κ B signaling pathway (see Table 1 and Table S1 in the supplemental material for abbreviations). Analysis of the entire data set from Table S1 in the supplemental material was done using Ingenuity Pathway Analysis software: pink/red, upregulation (P value, ≤ 0.05); green, downregulation (P value, ≤ 0.05); white, unchanged; and blue/gray, P value ≥ 0.05 .

when infected monocyte extravasation and differentiation occur.

The importance of other cellular signaling pathways during the early stages of infection cannot be ignored. Although the downregulation of expression of members of the mitogen-activated protein kinase (MAPK) pathways suggest that their chronic activation may not be critical to viral replication, rapid activation may be required for the initial dissemination of monocytes from blood into peripheral tissue. For example, induction of matrix metalloproteinase 1 (MMP-1), which is essential for monocyte migration through the basement mem-

branes, was not dependent on NF- κ B or PI(3)K activity in our studies. MMP-1 promoter activity is regulated by the extracellular signal-regulated kinase signaling pathway through the transcription factors Ets1 and Fli1 (4). In monocytes, the related MAPK family member p38 is rapidly and transiently activated following infection (17), suggesting that members of the MAPK pathway may be responsible for the induction of MMP-1 gene expression following infection.

The activation of cellular pathways rather than the de novo expression of viral genes is likely the major mechanism for the rapid monocyte gene regulation following HCMV infection,

since viral gene expression is not seen until several weeks postinfection (13). Treatments of monocytes with UV-inactivated HCMV and the major glycoprotein gB exhibited similar effects (17), indicating that receptor-ligand interactions initiate the cellular activation following HCMV infection of monocytes. Together, these studies point out that because HCMV binding to cognate cellular receptors triggers the induction of NF- κ B and PI(3)K activity in monocytes, it also triggers the induced proinflammatory state exhibited by increased cytokine/chemokine secretion, motility, endothelial adhesion, transendothelial migration, and monocyte-to-macrophage differentiation. Indeed, our results here suggest that HCMV utilizes cellular signal transduction pathways to ensure proper monocyte programming for viral dissemination and replication.

We have previously proposed the following model of HCMV dissemination (13): (i) epithelial cells of the host become infected by contact with HCMV-containing bodily fluids, (ii) HCMV replicates and spreads to monocytes in the peripheral blood by an unknown mechanism, (iii) primary infection of monocytes promotes migration into host organ tissue, and (iv) infection promotes differentiation into replication-permissive macrophages. This study provides insight into how HCMV hijacks the cellular inflammatory response as a mechanism to promote viral persistence that is consistent with our model. Initial infection results in the rapid activation of peripheral blood monocytes via the action of the NF- κ B and PI(3)K signaling pathways (14, 15, 17). Overall, this global transcriptome analysis of HCMV-infected monocytes provides additional evidence that the NF- κ B and PI(3)K signaling pathways serve as critical control points in HCMV-induced monocyte activation.

Microarray accession number. The GEO accession number for these data is GSE9601.

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