Published in final edited form as:
J Neuroimmune Pharmacol. 2010 September ; 5(3): 418–427. doi:10.1007/s11481-010-9212-0.

A Common Neuronal Response to Alphaherpesvirus Infection

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Abstract

Alphaherpesviruses are a subfamily of the *Herpesviridae* that can invade the nervous system and establish either lytic or latent infections. The establishment of latent infection can occur only in neurons, indicating a unique virus–host interaction in these cells. Here, we compare results from seven microarray studies that focused on the host response of either neural tissue or isolated neurons to alphaherpesvirus infection. These studies utilized either herpes simplex virus type 1 or pseudorabies virus as the infectious agent. From these data, we have found common host responses spanning a variety of infection models in different species, with different herpesvirus strains, and during all phases of infection including lytic, latent, and reactivation. The repeated observation of transcriptional effects on these genes and gene families indicates their likely importance in host defenses or the viral infectious process. We discuss the possible role of these different genes and genes families in alphaherpesvirus infection.

Keywords

alphaherpesviruses; HSV-1; virus; latency; microarray; neurons; gene expression

Introduction

Alphaherpesviruses are a subfamily of the *Herpesviridae* that include three human pathogens: varicella-zoster virus and herpes simplex virus types 1 and 2 (HSV-1 and HSV-2), and important agricultural pathogens such as pseudorabies virus (PRV) and bovine herpesvirus 1 (BHV-1; Flint et al. 2009). These alphaherpesviruses share a common virion structure, several homologous genes, and the ability to establish a lifelong latent infection. Most alphaherpesviruses share a signature pathway of infection: they establish a primary infection at a mucosal or epithelial surface and then leave that site by invading the peripheral nervous system (PNS), where they establish a reactivatable latent infection (Roizman et al. 1993). Although infection of nonneuronal cells results almost exclusively in a productive, lytic infectious cycle, infection of PNS neurons in the natural host frequently leads to a quiescent, latent viral state. This finding indicates that features of the host cell influence the decision to establish a productive or latent infection. Likewise, since a variety of external stressors can induce reactivation from latency, it is likely that host factors play a role here as well.

Recent studies indicate that host gene expression can affect the outcome of alphaherpesvirus infection. Transcription of cyclooxygenase 2 (Cox-2), a key prostaglandin biosynthesis gene, is induced after PRV infection (Ray and Enquist 2004). This induction is essential for

Electronic supplementary material The online version of this article (doi:10.1007/s11481-010-9212-0) contains supplementary material, which is available to authorized users.

PRV replication, as blockade of Cox-2 function by exogenous inhibitors reduces PRV growth in vitro (Ray et al. 2004). Cox-2 inhibitors also impair replication of the betaherpesvirus cytomegalovirus in cultured fibroblasts (Zhu et al. 2002) and reduce transcription of a latency-associated gene in the gamma herpesvirus Kaposi's sarcoma herpesvirus (KSHV) in vitro (Sharma-Walia et al. 2006). Work from the Casanova lab showed that a single gene can be essential for natural immunity to HSV-1 in the central nervous system. The occurrence of HSV-1-induced encephalitis in otherwise healthy children is correlated with defects in either Toll-like receptor 3 or UNC-93B (Casrouge et al. 2006; Zhang et al. 2007). Other work in mice has demonstrated that deletion of early growth response 1 (Egr1) prevents lethality by HSV-1 infection (Chen et al. 2008). These examples support the contention that host gene expression can affect the progression of infection, with either beneficial or injurious effects for the host. These examples suggest new treatment options for HSV-1 infection, emphasizing the importance of identifying other host gene products involved in viral infection.

Large-scale survey techniques such as DNA microarrays have been used to identify host responses to pathogen invasion. Microarrays monitor the effects of pathogens on host gene expression on a broad scale; however, the comparison of results produced by these different studies is complicated by the wide variety of microarray platforms and host models used. In 2005, Jenner and Young conducted a meta-analysis of 32 published studies that involved 77 different host–pathogen interactions (Jenner and Young 2005). They examined the host response to a wide spectrum of pathogens, including bacteria, viruses, and related stimuli. Despite the disparate microorganisms, viruses, host cell types, and methodological systems, they found a core group of immune-responsive genes that were activated in a majority of these host–pathogen interactions. We found that this comparative approach can be useful for the more focused case of how alphaherpesviruses interact with the nervous system, and particularly, with neurons.

In the last few years, several experiments have examined the host response to alphaherpesviruses infection using microarrays. Here, we compare results from seven studies that focused either on neural tissue or isolated neurons as they responded to herpesvirus infection (Table 1). These studies address different stages of viral infection. Some test productive infection, whereas others monitor latency or early stages of reactivation. These studies were carried out in a variety of model organisms and two in vitro neuronal cell models. We hypothesize that any changes in expression that are shared by these disparate models represent noteworthy candidates for common neural response genes.

Comparing across published studies

We first extracted the genes described by the authors of these seven studies as showing a twofold or greater change in expression in response to infection. Two studies did not provide any data on decreases in gene expression (Paulus et al. 2006; Clement et al. 2009). The trend in all studies was the observation of more increases than decreases in gene expression. This trend may be due either to sensitivity of measurements or to differences in expression analysis methods. Using online database annotation tools, we cross-referenced the published gene identifiers to obtain the mouse Entrez Gene ID as a common identifier for each gene (Dennis et al. 2003; Diehn et al. 2003; Maglott et al. 2007). We selected mouse for the common organism identifier because a large number of herpesvirus studies are conducted in this species. This mapping and compilation yielded a total of approximately 650 genes (sources listed in Table 1).

We next searched for common themes among these host responses to herpesvirus infection. Since the number of genes provided by most of these studies is small, we did not limit our

query to those genes that matched exactly across multiple studies. Instead, we focused on commonalities across gene families. For instance, we considered effects on multiple subunits of the same protein (e.g., AP complex subunits) or different members of the same protein family (e.g., multiple kinesins) to indicate a likely importance of these families in the host response to herpesvirus infection. We found 50 gene families that were affected in two or more studies (Table 2; individual gene names provided in Supplemental Table 1). We found 15 genes that did match exactly across two or more studies (Table 3). For example, the chemokine ligand CXCL12 was noted in two studies (Table 3; Prehaud et al. 2005;Clement et al. 2008), whereas the CXCL gene family members CXCL12, CXCL14, and CXCL9 are found in a total of four studies (Table 2;Kramer et al. 2003;Kent and Fraser 2005;Prehaud et al. 2005;Clement et al. 2008).

When assessing changes in gene expression observed by large-scale microarray studies, it is important to keep in mind several caveats. First, the diversity of platforms used to measure changes, and the different approaches used to analyze these data, mean that it is extremely difficult to find exact transcript matches across different platforms and experimental studies. Different microarray platforms may hybridize a slightly different segment of a given transcript, and thus may measure one or all splice variants, or be more or less sensitive than another platform. Second, when comparing lists of affected genes or pathways from multiple studies, any summaries of pathways *not* affected in a given study must be considered carefully. The failure to observe an effect on a given pathway is not conclusive unless the study has observed an effect on this pathway in another situation, so that their ability to successfully measure this pathway can be confirmed. As an example, Prehaud et al. (2005) used the human NT-2 neuronal cell line as a model system to study neuronal responses to HSV-1 and rabies virus infection. They observed that while approximately 25% of the neuronal gene expression responses to rabies virus were immune-related, only 5% of the responses to HSV-1 infection were immune-related. This parallel approach provides confidence in the limited immune response of these neurons to HSV-1, since an abundance of immune response transcripts was elicited by rabies virus infection.

Exact gene family members may not match across multiple studies because we have mapped these results across infection models in multiple species (human, rabbit, mouse, and rat), with five different herpesvirus strains, during different phases of infection (productive, latent, and reactivation), and in both in vivo and in vitro studies. However, we gained further insight by examining the overlap between subsets of these data more closely. For instance, in vitro studies offer the advantage of studying a pure population of neuronal cells. On the other hand, the single-cell-type isolation of in vitro systems is artificial, and the possibility must be considered that responses observed in vitro would not occur in the presence of normal intercellular interactions in vivo. In contrast, animal models more fully encompass the nervous system's true state, in that infected neurons interact closely with glia and microglia, as well as infiltrates from the adaptive immune system. However, in vivo models have the confounding issues of asynchronous infection, variability in the degree of immune cell infiltration, and potential for decreased detection sensitivity because expression changes are blurred by multiple cell types in the tissue sample. Therefore, cases where a gene family's expression level has been observed to change both in vivo and in vitro strongly imply a cell autonomous neuronal response.

Specific gene families of interest

Immune response

The largest category of gene families affected during the host response to alpha-herpesvirus infection contains immune-related genes. All but two of the studies reviewed here used whole nervous system tissues where systemic immune involvement was likely. We have

previously mentioned the multi-pathogen meta-analysis by Jenner and Young (2005), where they found a core group of approximately 500 genes whose expression comprised the common host response to infection by any pathogen. The majority of studies summarized by Jenner and Young used nonneuronal cells, so membership in the common host response group suggests a broad function. We compared the genes listed in Table 2 with this common host response list and found 25 in common (marked in Tables 2 and 3; also listed in Supplemental Table 1). Most of the immune response gene families summarized in this review fall into this group, suggesting that immune-related gene expression changes are not particularly unique to herpesvirus infection, and that they likely comprise part of the generalized host response to pathogen infection.

The expression of immune response genes measured by all studies was universally increased. Only a subset of these immune responses was found in both in vivo and in vitro studies (subheading “Immune response—neuronal” in Table 2), particularly multifunctional signaling genes such as the CXC chemokine ligands and receptors. In contrast, genes more related to antigen presentation or humoral immunity, such as Fc receptors and complement components, were found only in the in vivo neural tissue models. This observation may indicate that changes in expression of these genes are occurring in infiltrating immune cells rather than in neurons. For example, the integrins found in this group include both the alpha-M and beta2 partner subunits; these genes are not known to be expressed in neurons but rather act together to form the Mac-1 receptor found on microglia and lymphocytes (Rock et al. 2004;Kinashi 2005).

Expression of interferon regulatory factor 1 (IRF1), another immune response gene, increased during all phases of infection. IRF1 is part of Jenner and Young’scommon host response group, and it appears in both in vivo and in vitro neural studies. This gene is a member of the larger IRF family of transcription factors, which induce interferon-stimulated genes in response to interferon signaling. IRF1 has been previously confirmed as being expressed in neurons, although its downstream effects may be more limited than in nonneuronal cells (Thomas et al. 1997). Based on work in nonneuronal cells (Collins et al. 2004; Lin et al. 2004), it seems likely that the increased IRFF1 and IRF7 expression found in these studies (Kent and Fraser 2005; Prehaud et al. 2005; Paulus et al. 2006) most likely reflects part of the host defensive response to herpesvirus infection.

CCAAT enhancer-binding proteins (C/EBP) are bZIP transcription factors that function as both homo- and heterodimers. C/EBP alpha has been proposed to play a supportive role in BHV latency and reactivation (Meyer et al. 2007; Meyer and Jones 2009), as well as during infection by the gamma–herpesviruses Epstein-Barr Virus and KSHV (Wang et al. 2003; Wu et al. 2004). Those studies found that C/EBP alpha stimulated the progression of these infections. In the studies reviewed here, expression of the C/EBP family members beta, delta, and epsilon was induced in both in vitro and in vivo models of productive herpesvirus infection, an effect found for both PRV and HSV-1 infections (Prehaud et al. 2005; Paulus et al. 2006). The fact that C/EBP proteins can heterodimerize suggests that these C/EBP family members may act similarly to C/EBP alpha and actually enhance herpesvirus infection in neurons, rather than acting as part of a host defensive response.

Signal transduction

The expression of most signal transduction genes found among these studies was reduced. This finding is noteworthy given the limited number of genes whose expression decreased at all. This group includes several major families of kinases and phosphatases, including calcium/calmodulin-dependent and cAMP-dependent kinases, PKC family members, and the PP2 family of phosphatases. Perhaps most striking among the downregulated signal transduction members, however, are the large subgroup of G-protein-associated signal

transduction partners: four protein families including eight different members, almost exclusively downregulated across all infection paradigms and species (for a review of this pathway, see Tybulewicz and Henderson 2009). Regulator of G-protein signaling 4 (Rgs4) is a particularly well-known member of this group, since it has been implicated as a potential marker of schizophrenia and is highly expressed in the brain (Ding et al. 2007;Lang et al. 2007). Rgs4, like other Rgs proteins, negatively regulates G-protein signal transduction cascades, via its GTPase activity on the G-alpha (i) and G-alpha(o) subunits (Watson et al. 1996). The G-alpha(o) subunit is actually one of two G-alpha types listed in Table 2; it is downregulated during HSV-1 latency (Clement et al. 2008). Rgs4 has also been described as affecting inward-rectifying potassium channels, which is potentially an additional means by which herpesvirus infection may affect intracellular signaling in neurons (see “Other” section below; Jaen and Doupnik 2006). Rho GTPase interactions with the actin cytoskeleton and actin filament formation have been noted to play important roles in herpesvirus entry and replication (Favoreel et al. 2007). The finding of downregulation of three Rgs types, two Rho GTPase-activating proteins (GAPs), and the Rho GDP dissociation inhibitor adds further strength to the hypothesis that this pathway is important during all phases of herpesvirus infection.

The one exception to the overall reduced expression of signal transduction proteins across these studies is the gene family of dual specificity phosphatases (Dusps). Dusps act on members of the mitogen-activated protein (MAP) kinase signaling pathway. Expression of Dusps 1, 2, and 5 was increased in both lytic and latent models, using PRV and HSV-1 stimuli, in isolated neuronal cells and in whole animals (Paulus et al. 2006; Danaher et al. 2008). All three of these Dusp family members are also part of Jenner and Young’s common host response group, indicating their overarching function in the general host response to pathogens (Jenner and Young 2005). The Us2 protein of PRV interacts directly with ERK (Lyman et al. 2006), a member of the MAPK pathway modulated by Dusps. The Dusps are involved in immune responses and inflammation (reviewed in Lang et al. 2006; Salojin and Oravecz 2007), suggesting that their increased expression reflects the host defensive responses to herpesvirus infection.

Transcription regulators

Increased expression of several families of transcriptional regulators was common to both in vivo and in vitro studies (Table 2). Interestingly, transcriptional regulators constitute the largest group of exact gene matches found across these studies (Table 3), suggesting a more conserved role for these gene products. Because most of these proteins are known to regulate transcription of several intracellular pathways, it is difficult to predict their exact role during viral infection. However, most of these gene families fit into one of two groups: genes known to regulate developmental processes and differentiation and genes that regulate stress responses.

Several members of the stress responses group of transcription regulators are known to be involved in HSV-1 infection. Egr1 and Egr2 repress the HSV-1 latency-associated transcript (LAT) promoter (Tatarowicz et al. 1997). In addition, Egr1 represses the promoters of HSV-1 immediate-early genes ICP4 and ICP22 (Bedadala et al. 2007). In mouse studies, Egr-1 expression was increased in HSV-1-infected brains (correlating with these data), and Egr-1 enhanced viral replication in embryonic fibroblasts (Chen et al. 2008). Furthermore, Egr-1 knockout mice show reduced mortality by HSV-1, indicating the significant role of Egr1 in the viral life cycle (Chen et al. 2008).

Signal transducers and activators of transcription (Stats) convert intracellular signals from interferon receptors to the nucleus and activate transcription of interferon-stimulated genes. Several studies suggest that STAT1, which is also part of Jenner and Young’s common host

response group, is involved in HSV-1 infection. Fujii and colleagues found an inhibitory effect of HSV-1 proteins on phosphorylation of Stat1, which in turn dramatically suppressed both type I and type II interferon-inducible genes during the early stage of HSV-1 infection (Yokota et al. 2001). A similar inhibition of Stat1 phosphorylation occurs during PRV infection (Brukman and Enquist 2006). Stat1 was also found to bind the HSV-1 LAT promoter (Kriesel et al. 2004). These results indicate complex interactions between the virus and host transcription activators.

Intracellular structure and transport

Alphaherpesviruses use the host cytoskeleton for efficient entry, replication, and egress. Multiple viral cargoes must be moved to appropriate sites within infected cells, in a temporally relevant way (reviewed by Lyman and Enquist 2009). Therefore, one might anticipate regulation of host cytoskeletal proteins and molecular motors during the viral life cycle. We observe that expression of most of the gene families found in this group is increased during productive infection and decreased during latency (Table 2).

The anterograde transport of alphaherpesvirus in axons is dependent on kinesin microtubule motor proteins (Smith et al. 2001; Diefenbach et al. 2008). However, many kinesin family members exist, and thus far, only KIF1A has been found to interact with an HSV protein (HSV-2 UL56) in vitro (Koshizuka et al. 2005). Expression of KIF1A, a motor protein responsible for the axonal transport of synaptic vesicle precursors, is not increased in any the studies we compare here. Instead, expression changes were noted for Kif2C, 3C, 5A, and 5C (Kent and Fraser 2005; Prehaud et al. 2005; Clement et al. 2008). No evidence is yet available on their potential interactions with viral cargo.

Proteolysis

Many proteolytic enzyme genes and their gene families are increased in expression across these studies, including cathepsins, proteasome subunits, matrix metallopeptidases, and serine peptidase inhibitors. Among these, only the cathepsins, a large family of lysosomal proteases, have increased expression during all phases of infection (Table 2). Cathepsin B, although not one of genes expressed in the studies noted here, has been previously noted for its role in processing of the herpesviral origin binding protein (Link et al. 2007). Cathepsin D, which was found to be upregulated in both early and latent infection by Kramer et al. (2003), is known for its neuronal expression. Its absence mimics a neurodegenerative syndrome (Shacka et al. 2007). Cystatins, the endogenous inhibitors of cathepsins, have been demonstrated to block HSV-1 replication (Bjorck et al. 1990;Peri et al. 2007). The multifunctional cathepsin family of proteases, of which four members were found across four independent infection models (Kramer et al. 2003;Kent and Fraser 2005;Clement et al. 2008;Danaher et al. 2008), could potentially be important for processing of herpesviral proteins or in maintaining the integrity of infected neurons, making it a useful potential therapeutic target.

Extracellular matrix-related

The expression of most extracellular matrix gene families found in common across these studies is increased. Only the collagen protein family has reduced expression. In combination with the increases in metallopeptidase expression (ADAM and MMP proteins; Table 2), we suggest that during viral infection, collagen expression decreases, and collagenase function is enhanced. Previous work suggests that collagen can restrict HSV-1 infectivity (McKee et al. 2006;Kolodkin-Gal et al. 2008), suggesting that these changes may be beneficial to the virus.

Other

Several gene families did not fit into the standard categories. The increased expression of potassium voltage-gated channel proteins stands out in three studies spanning both in vivo and in vitro models (Kramer et al. 2003; Prehaud et al. 2005; Danaher et al. 2008). This observation is striking since sodium channels are the only neuronal ion channels previously associated with alphaherpesvirus infection (Storey et al. 2002; Garry et al. 2005). As noted above, Rgs4 may affect non-voltage-gated potassium channel function, providing two potential means of affecting potassium flux in infected neurons.

Future directions

The genes and gene families highlighted here have been identified from data gathered using a variety of infection models in different species, with different strains, and during all phases of infection. Not surprisingly, the conservation of effects is not at the level of individual genes, but instead at the level of pathways or families of genes that are likely to be involved in the progression of infection. What we cannot predict from these observations is whether expression of these genes represents the host's defensive response to infection or reflects virus-induced changes in the host repertoire that are beneficial to infection. Initial work provides some insight into these possibilities. For instance, Egr1 and C/EBP transcription factors may act to enhance infection, while Stat1 and many of the immunity genes may be activated by infected cells in an attempt to combat infection. Further work at the level of individual genes or pathways will be needed to understand these effects for the lesser-known genes found in common across these studies. However, the convergence of data across multiple studies, species, and virus strains indicates that the genes listed in Tables 2 and 3 are good candidates for exactly this sort of gene-by-gene examination, since knockout mice are available for many of them. Testing these mice with an in vivo infection model, for effects on lytic, latent, and reactivating phases of infection, should be revealing.

The comparison of data across both in vitro and in vivo models also provides useful insights for future experimentation. To date, there have been only two microarray studies published using any type of in vitro neuronal infection model, one of productive infection (Prehaud et al. 2005) and one of a quiescent or latency model (Danaher et al. 2008). The only deficit in these studies lies in the use of immortalized cell lines, rather than primary cells, as a neuronal model. Although they resemble neurons both morphologically and functionally, these cell types have chromosomal abnormalities and potentially other less-apparent changes in their signaling pathways. It would thus be important to compare these studies with infection of primary neuronal cultures.

An important consideration with all studies of gene expression is that these data do not relate RNA levels to protein levels within cells. Post-transcriptional regulation, such as alternative splicing, delayed translation, and mRNA degradation, can all affect the protein level that results from a given transcriptional output. While proteomic analyses have not yet been applied to infected neurons, early results from alternative models (Loret et al. 2008; Skiba et al. 2008; Santamaria et al. 2009) suggest that these techniques will likely be fruitful for gaining comprehensive data about proteins levels in infected cells.

In the future, neuronal host proteins whose functions have been subverted to enhance or even be required for herpesvirus infection may be potential therapeutic targets. Because of the role of neurons as the long-term reservoir for latent alphaherpesviruses, drugs that target neuronal proteins affecting latency or reactivation—provided that they did not interfere with normal neuronal function—may provide improved palliative therapies, or even raise the possibility of a cure for herpesvirus infection. Targeting host proteins may help to avoid the rapid evolution of drug resistance that occurs when viral proteins are the primary drug

target. Although these ideas may take years to reach the point of useful therapies or treatments, these initial studies of the host neuronal response to infection provide much needed data to fuel these research ideas.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank C. Préhaud and M. Lafon for sharing additional data not shown in their prior publication. MLS is funded by the New Jersey Commission on Spinal Cord Research and NIH P40 RR 018604. OK is funded by Human Frontier Science Program. LE acknowledges support from NIH grants R37 NS 033506, R01 NS060699, P40 RR 018604, and P50 GM071508.

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Table 1

Large-scale gene expression studies examining neuronal or nervous system responses to alphaherpesvirus infection

Type of infection	Cell or tissue type	Species	α-Herpesvirus (strain)	Time-points p.i.	No. of genes listed	Array type	Reference[a]
Productive	NT2-N neuronal cell line	Human	HSV (KOS)	18 hours p.i.	396 (286 up)	Affymetrix human U133A-B	1
Productive	Cerebellum and hypothalamus	Rat	PRV (Becker)	48 and 60 hours p.i.	33 (33 up)	Affymetrix rat U34 A	2
Productive and latent	Trigeminal ganglia	Mouse	HSV (KOS)	3, 10, and 30 days p.i.	52 (44 up)	Non-commercial nylon array	3
Latent	Trigeminal ganglia	Rabbit	HSV (McKrae)	30 days p.i.	44 (17 up)	Agilent custom rabbit	4
Latent (quiescent)	PC12 neuronal cell line	Rat	HSV (17+)	17 days p.i.	42 (28 up)	Affymetrix rat 230A	5
Latent and reactivation	Trigeminal ganglia	Mouse	HSV (F)	30 days p.i.+30 min. react.	56 (35 up)	Clontech Atlas mouse 1.2 cDNA	6
Reactivation	Trigeminal ganglia	Mouse	HSV (17+)	28 days p.i., and 1 hour stress	26 (26 up)	Affymetrix mouse 430 2.0	7

p.i. post-infection

[a]References: (1) Prehaud et al. (2005), (2) Paulus et al. (2006), (3) Kramer et al. (2003), (4) Clement et al. (2008), (5) Danaher et al. (2008), (6) Kent and Fraser (2005), and (7) Clement et al. (2009)

Table 2

Gene families noted in two or more studies of neuronal or nervous system responses to alphaherpesvirus infection

Gene family	No. of genes[a]	Productive	Latency	Reactivation	References[b]
Immune response—immune system					
CD antigen	9 (2)	↑	↑	↑	1, 3, 6, 7
Chemokine (C-C motif) ligand	2 (2)			↑	6, 7
Complement component	6 (1)	↑	↑		2, 3
Fc receptor, IgG, low affinity	2	↑	↑	↑	3, 6
Histocompatibility 2 (H2 locus)	3 (1)	↑	↑		2, 3, 4
Immunity-related GTPase family M member	2	↑	↑	↑	2, 6
Immunoglobulin chain	3		↑	↑	4, 7
Integrin	3	↑	↑	↑	1, 3, 6
Immune response—neuronal					
CCAAT/enhancer-binding protein (C/EBP)	3	↑			1, 2
Chemokine (C-X-C motif) ligand	3 (1)	↑	↑	↑	1, 3, 4, 6
Chemokine (C-X-C motif) receptor	2 (1)	↑	↑		1, 3
Interferon regulatory factor (IRF)	2 (2)	↑	↑	↑	1, 2, 6
Interleukin (IL)	2 (2)	↑	↑		1, 4
Signal transduction—kinases/phosphatases					
Calcium/calmodulin-dependent protein kinase (CaMK)	2		↓		4, 6
Dual specificity phosphatase (DUSP)	2 (3)	↑	↑		2, 5
Protein kinase C (PKC) superfamily	2	↓	↓		1, 6
Protein kinase, cAMP-dependent regulatory (PKA family)	2		↓	↓	4, 6
Protein phosphatase (PP2) family	3		↓	↓	3, 5, 6
Signal transduction—G proteins					
Guanine nucleotide binding protein, alpha	2	↓	↓		1, 4
Regulator of G-protein signaling (Rgs)	3	↑ ↓	↓	↓	1, 3, 6
Rho GTPase-activating protein (RhoGAP)	2		↓		4, 5
Transcription regulators—developmental					
Chromobox homolog	2	↑			1, 2
Delta-like 1	2	↑	↑	↑	1, 6, 7

Gene family	No. of genes[a]	Productive	Latency	Reactivation	References[b]
Kruppel-like factor	3 (1)	↑	↑		1, 5
Zinc finger protein of the cerebellum (Zic)	3	↑	↓		1, 6
Transcription regulators—stress response					
Early growth response (Egr)	3	↑	↑		1, 5, 6
FBJ osteosarcoma oncogene (Fos)	3	↑	↑	↑	2, 5, 7
Signal transducer and activator of transcription (STAT)	2 (2)	↑	↑	↑	1, 3, 6
Intracellular structure and transport					
Adaptor-related (AP) protein complex	3	↑↓	↓		1, 4
Kinesin family (Kif) member	4 (1)	↑	↓	↑↓	1, 4, 6
Neurofilament	2	↑		↓	1, 6
Tubulin, beta (TUBB)	2		↓	↓	4, 6
Proteolysis					
Cathepsin	4 (1)	↑	↑	↑	3,4,5,6
Proteasome subunit, beta type	2 (2)	↑↓	↑		1, 3
Serine (or cysteine) peptidase inhibitor (serpin)	3	↑		↑	1, 6
Extracellular matrix-related					
A disintegrin and metallopeptidase domain (ADAM)	3	↑	↑		1, 3, 5
Collagen	6	↑↓	↑↓		1, 3, 4
Keratin	2	↑		↑	1, 7
Matrix metallopeptidase (MMP)	4 (1)	↑	↑		1, 5
Metabolic pathways					
Carnitine palmitoyltransferase 1	2	↑	↓		1, 4
Mannosidase 2, alpha	2	↑↓	↑		1, 3
NADH dehydrogenase	2	↓	↑		1, 4
Pyruvate dehydrogenase kinase (Pdk)	2	↑	↓		1, 5
Ribosomal protein	6	↓	↑↓	↑	1, 4, 6
Others					
Ankyrin repeat domain	2		↑		4, 5
Catenin (cadherin associated protein), alpha	2	↓		↓	1, 6
Cysteine-rich secretory protein (CRISP)	2	↑	↑		1, 4
Nuclear receptor subfamily	4 (1)	↑			1, 2

Gene family	No. of genes[a]	Productive	Latency	Reactivation	References[b]
Potassium voltage-gated channels	4	↑	↑↓		1, 3, 5
RNA binding motif protein	2	↑	↑		1, 5

Arrows indicate increases (↑) or decreases (↓) in observed gene expression during specified stages of infection (productive, latency, or reactivation).

[a] Parentheses indicate number of gene family members observed here that are also part of the generalized host response to pathogens described by Jenner and Young (2005)

[b] References: (1) Prehaud et al. (2005), (2) Paulus et al. (2006), (3) Kramer et al. (2003), (4) Clement et al. (2008), (5) Danaher et al. (2008), (6) Kent and Fraser (2005), and (7) Clement et al. (2009)

Table 3

Exact gene matches found in more than one study of neuronal or nervous system responses to alphaherpesvirus infection

Gene symbol	Gene name	Mouse Entrez ID	Productive	Latency	Reactivation	References[b]
Immune response						
'Cxcl12	Chemokine (C-X-C motif) ligand 12	20315	↑	↑		1, 4
'Irf1[a]	Interferon regulatory factor 1	16362	↑	↑	↑	1, 6
'Il2rg	Interleukin 2 receptor, gamma chain	16186	↑	↑	↑	3, 6
Signal transduction						
'Gh	Growth hormone	14599		↓	↑	3, 7
'Itgb2	Integrin beta 2	16414	↑	↑		1, 3
'Rgs4	Regulator of G-protein signaling 4	19736		↓	↓	3, 6
'Arhgdib	Rho, GDP dissociation inhibitor (GDI) beta	11857	↓	↑	↑	1, 6
Transcription regulators						
'Btg2[a]	B-cell translocation gene 2, antiproliferative	12227	↑	↑		2, 6
'Dlk1	Delta-like 1 homolog (*Drosophila*)	13386		↑	↑	6, 7
'Egr1	Early growth response 1	13653	↑	↑		1, 5
'Egr2	Early growth response 2	13654	↑	↑		1, 5
'Fos	FBJ osteosarcoma oncogene	14281	↑	↑		2, 5
'Klf5	Kruppel-like factor 5	12224	↑	↑		1, 5
'Stat1[a]	Signal transducer and activator of transcription 1	20846	↑	↑	↑	3, 6
Metabolic pathways						
'Idi1	Isopentenyl-diphosphate delta isomerase	319554	↑	↑		2, 5

Arrows indicate increases (↑) or decreases (↓) in observed gene expression during specified stages of infection (productive, latency, or reactivation).

[a] These genes are part of the generalized host response to pathogens described by Jenner and Young (2005)

[b] References: (1) Prehaud et al. (2005), (2) Paulus et al. (2006), (3) Kramer et al. (2003), (4) Clement et al. (2008), (5) Danaher et al. (2008), (6) Kent and Fraser (2005), and (7) Clement et al. (2009)