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Gene Expression Profiles of HIV-1-Infected Glia and Brain: Toward Better Understanding of the Role of Astrocytes in HIV-1-Associated Neurocognitive Disorders

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Abstract

Astrocytes are the major cellular component of the central nervous system (CNS), and they play multiple roles in brain development, normal brain function, and CNS responses to pathogens and injury. The functional versatility of astrocytes is linked to their ability to respond to a wide array of biological stimuli through finely orchestrated changes in cellular gene expression. Dysregulation of gene expression programs, generally by chronic exposure to pathogenic stimuli, may lead to dysfunction of astrocytes and contribute to neuropathogenesis. Here, we review studies that employ functional genomics to characterize the effects of HIV-1 and viral pathogenic proteins on cellular gene expression in astrocytes in vitro. We also present the first microarray analysis of primary mouse astrocytes exposed to HIV-1 in culture. In spite of different experimental conditions and microarray platforms used, comparison of the astrocyte array data sets reveals several common gene-regulatory changes that may underlie responses of these cells to HIV-1 and its proteins. We also compared the transcriptional profiles of astrocytes with those obtained in analyses of brain tissues of patients with HIV-1 dementia and macaques infected with simian immunodeficiency virus (SIV). Notably, many of the gene characteristics of responses to HIV-1 in cultured astrocytes were also altered in HIV-1 or SIV-infected brains. Functional genomics, in conjunction with other approaches, may help clarify the role of astrocytes in HIV-1 neuropathogenesis.

Keywords

astrocytes; microarrays; gene expression profiling; HIV-1; HAD; brain; neurobiology; neurodegeneration; innate immunity

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Introduction

Neuroglia are non-neuronal brain cells consisting of two broad cell categories, microglia and macroglia (Skoff and Knapp 1995; Streit 1995). Microglia originate in bone marrow and represent resident macrophages in the brain; they play major roles in innate immune responses and neuroinflammation (Streit 1995). Macroglia, similar to neurons, originate from neuroectoderm, and they include three major cell types, oligodendrocytes, radial glia, and astrocytes (Skoff and Knapp 1995). Oligodendrocytes are highly specialized glial cells responsible for myelinating axons. Radial glia serve as precursor cells during neurogenesis and, as Bergmann glia, modulate synaptic plasticity in adult brain (Skoff and Knapp 1995). Astrocytes are the most abundant, heterogeneous, and functionally versatile type of neuroglia (Schubert 1984; Skoff and Knapp 1995; Davies et al. 2000; Bachoo et al. 2004). Their roles range from structural and metabolic support in the brain, maintenance of brain homeostasis, responses to brain injury and pathogens, regulation of synaptic activity and neural signaling, to participation in brain development (reviewed in Benveniste et al. 1998; Bezzi and Volterra 2001; Danbolt 2001; Farina et al. 2007; Seth and Koul 2008; Silver and Steindler 2009).

This review focuses on the applications of functional genomics to the study of astrocyte biology in the context of HIV-1 infection and HIV-1-associated neurocognitive disorder (HAND). HAND (Antinori et al. 2007) and its most severe manifestation HIV-1 dementia (HAD) persist at a high rate in spite of the increasingly common use of antiretroviral therapies (McArthur 2004; Anthony et al. 2005; Ellis et al. 2007). Although the core pathophysiological defects of HAD are neuronal damage (Masliah et al. 1992; Everall et al. 1993; Navia and Price 1998), neurons rarely show evidence of HIV-1 infection (Wiley et al. 1986; Takahashi et al. 1996; Torres-Muñoz et al. 2001a). HIV-1 replicates in the brain in microglia and infiltrating macrophages (Gartner et al. 1986; Genis et al. 1992), and virus genome and proteins can also be found in a small and variable fraction of astrocytes in vivo, particularly in advanced brain disease (Saito et al. 1994; Tornatore et al. 1994; Ranki et al. 1995; Takahashi et al. 1996; An et al. 1999; Trillo-Pazos et al. 2003). The consensus view is that HIV-1 can be neuropathogenic through neurotoxic viral proteins and cellular toxins and inflammatory mediators secreted by HIV-1-infected macrophages and microglial cells, particularly in HIV-1 encephalitis (reviewed in Lipton and Gendelman 1995; Kolson and Pomerantz 1996; González-Scarano and Martín-García 2005). However, there is growing recognition that astrocytes also contribute to HIV-1-mediated neuropathogenesis, in several ways. First, astrogliosis, the presence of activated and hypertrophied astrocytes typically seen in HAD (Sharer et al. 1986; Budka 1991), may damage tissue by scarring. Second, as indicated above, HIV-1 can infect a small fraction of astrocytes in vivo. Productive infection of astrocytes with HIV-1 has significant effects on cell physiology in vitro (Kim et al. 2004; Cosenza-Nashat et al. 2006) and it associates with measurable neuropathology in a mouse model (Dou et al. 2006), suggesting that infected astrocytes, although infrequent, can have localized pathogenic effects. Third, astrocyte dysregulation resulting from exposure of the cells to HIV-1 particles, viral proteins, cytokines, and other mediators secreted by HIV-1-infected cells could have a broad impact on brain function (reviewed in Conant and Major 1998; Brack-Werner 1999; Wang et al. 2004). Studies in vitro indicate that many of these products significantly modulate astrocyte physiology, which in turn can alter essential interactions of astrocytes with other cells in the brain, particularly neurons. For example, exposure of cultured astrocytes to HIV-1, recombinant gp120, or viral transactivator Tat can induce several known mediators of neuropathogenesis, including inflammatory cytokines TNF-α and IL-1β, chemokines MCP-1 and IP-10, IL-6, and neurotoxin nitric oxide (Yeung et al. 1995; Conant et al. 1998; Lavi et al. 1998; Glabinski and Ransohoff 1999; Hesselgesser and Horuk 1999; Cota et al. 2000; Kutsch et al. 2000; McManus et al. 2000; Liu et al. 2002; El-Hage et al. 2005; Ronaldson and Bendayan 2006; Li et al. 2007). Binding

of HIV-1 or exogenous gp120 to astrocytes can reprogram cellular gene expression (Su et al. 2002, 2003; Galey et al. 2003; Wang et al. 2004), while the critical astrocyte function of glutamate uptake can be inhibited by exposure of the cells to HIV-1, gp120, Tat, or TNF-α (Benos et al. 1994; Fine et al. 1996; Wang et al. 2003; Zhou et al. 2004). Defective glutamate transport by astrocytes can result in neuronal death by excitotoxicity (Danbolt 2001). In another potential neurotoxic mechanism, recombinant gp120 was shown to induce Ca^{++}-dependent glutamate secretion by astrocytes and neuronal cell death in glial-neuronal co-cultures in a pathway involving signaling through CXCR4 and production of TNF-α (Holden et al. 1999; Bezzi et al. 2001). Because of their abundance and their many roles in the brain, it is clear that damage or dysregulation of astrocytes may significantly affect the normal physiological functioning of the nervous system.

Challenge of functional analysis of astrocytes

Astrocytes obtained from fetal or adult brain are morphologically and electrophysiologically diverse (Davies et al. 2000; Nakagawa and Schwartz 2004), and they show varied patterns of regional gene expression (Bachoo et al. 2004). It has been proposed that astrocytes stratify in vivo into subtypes or subpopulations distinguished by their anatomic location and brain region-specific functions (Bachoo et al. 2004). Molecular profiling of such astrocyte subgroups has only begun (Bachoo et al. 2004), and it may lead to a definition of astrocyte lineages such as those known for T cells. The lineages may vary in their responses to specific physiological and pathogenic stimuli. Laboratory studies of primary astrocytes generally use enriched GFAP-positive cell populations isolated from human fetal brain tissue or similarly prepared rodent fetal or neonatal astrocytes. These cultures represent cell pools originating from several anatomical regions of the brain, and they are morphologically and functionally heterogeneous. It is therefore difficult to ascertain whether responses of astrocytes to various stimuli in culture accurately reflect cellular changes under normal and pathological conditions in vivo. Some responses such as cell activation or secretion of chemokines (Conant et al. 1998; Cota et al. 2000; McManus et al. 2000; Ronaldson and Bendayan 2006) can be considered beneficial as part of normal neuroprotective function of the cells. Others such as inhibition of glutamate uptake or secretion of TNF-α (Benos et al. 1994; Holden et al. 1999; Bezzi et al. 2001; Wang et al. 2003) clearly have the potential to injure or impair neurons and contribute to the overall inflammatory environment in the brain. Another difficulty in unequivocal interpretation of astrocytic responses in culture is that some stimuli such as HIV-1 and gp120 can induce both neuroprotective (e.g., secretion of MCP-1) and neurotoxic astrocyte functions (e.g., glutamate uptake inhibition). Complicating matters further, as shown for HIV-1-infected macrophages (Lipton and Gendelman 1995; Kolson and Pomerantz 1996; González-Scarano and Martín-García 2005), unregulated stimulation of cells can cause excessive production of chemokines and cytokines, which rather than exerting their normal physiological functions can become neurotoxic and contribute to brain inflammation. These considerations underscore the challenges associated with functional evaluation of astrocytes in culture and extrapolation of the results to HIV-1 neuropathogenesis. Which of the HIV-1 effects on astrocytes in culture defines HIV-1-astrocyte interactions in vivo? Do stimuli such as gp120 or Tat induce different responses in different subpopulations of astrocytes? How can we distinguish normal from potentially pathogenic responses in the heterogeneous astrocyte populations in culture? Is it possible to identify molecular switches that can alter astrocyte physiology between normal and dysfunctional or harmful states?

One approach to attempt to analyze complex physiological responses of cells to external stimuli is by functional genomics, which permits objective and global determination of cellular gene expression profiles that are characteristic of normal and pathological conditions. Below, we describe applications of this approach to studies on astrocytes

exposed to HIV-1 and HIV-1 proteins in vitro. The molecular profiles of cultured astrocytes are compared to those obtained from brain tissue from patients with HIV-1 dementia and macaques infected with simian immunodeficiency virus (SIV) to better understand the role of astrocytes in HIV-1 neuropathogenesis.

Gene expression profiling of cells and tissues: general considerations

Highly regulated programs of cellular gene expression underlie most physiological functions of a cell, including cellular responses to extracellular cues. Functional genomics, namely, a genome-wide transcriptional analysis using microarray technology, is a highly efficient method for analyzing complex gene networks altered in one pathological situation, and it has become a standard tool for molecular studies in many diseases (for reviews, see Dufva et al. 2002; D'Agata and Cavallaro 2004; Glanzer et al. 2004; Cobb et al. 2005; Hoheisel 2006). One of the greatest advantages of the functional genomics method is that it permits objective analysis of expressional changes of thousands of genes in a single experiment. This provides a global view of complex, interconnecting, and often coordinate genomic responses (a gene expression profile) in well-defined cell or tissue populations.

There are multiple microarray approaches using different RNA detection platforms, experimental protocols, and statistical analysis methods (for example, see Churchill 2002; Scharpf et al. 2003; Hardiman 2004; Korn et al. 2004). This technological diversity may pose a difficulty in comparing microarray data from different studies, even when using the same source material (Tan et al. 2003; Jarvinen et al. 2004; Parmigiani et al. 2004). To assure compatibility of microarray information across different laboratories, the functional genomics community developed binding recommendations for methodological standardization of microarray experiments. "Minimum Information about a Microarray Experiment" (MIAME) lists specific experimental requirements that are essential for reproducing the microarray study by another laboratory (Brazma et al. 2001). A more recent standard called "Microarray Quality Control Project" has been developed by the Food and Drug Administration (Chen et al. 2007). Microarray experiment output consists of scans of labeled cDNA or cRNA samples hybridized to oligonucleotide or cDNA probes on a microchip. To obtain gene expression measures, scan data are subjected to probe-level statistical analysis, including quality control for hybridization and normalization across the array (Irizarry et al. 2003). This is followed by calculation of average fold changes in gene expression between systems of interest and controls and statistical significance analysis per gene. Depending on the cut-off significance values assigned to the experiment, a list from hundreds to thousands of "significantly changed" genes is generated.

One of the challenges of functional genomics is reduction of the large amounts of gene expression data generated by a microarray experiment to functional information, namely, to implicate specific genes and biological pathways in the biological process of interest. Although even large datasets can be examined gene by gene, a more practical approach is to employ a growing battery of bioinformatics methods that allow organizing data according to predetermined criteria (Kapetanovic et al. 2004). In complex biological systems, changes in gene expression occur coordinately rather than as independent events. Expression of many genes depends on the expression of others within biological and regulatory pathways. Most of microarray "data mining" programs take advantage of these functional relationships. One of the most commonly used is Gene Ontology (GO) analysis that allows classifying transcripts in well-defined biological pathways (Pavlidis et al. 2004; Werner 2008); popular GO programs include EASE (Hosack et al. 2003) and MAPPFinder (Doniger et al. 2003). Other approaches include clustering analysis that groups genes using different algorithms presenting similar expression patterns (Tibshirani et al. 1999). A similarity in gene expression can indicate a coordinate biological event (Kerr et al. 2008). A more recent

approach to identify significant but smaller changes in gene expression is the Gene Set Enrichment analysis, which follows behavior of clusters of functionally inter-related genes (gene sets) rather than individual genes (Kim and Volsky 2005; Nam and Kim 2008). Generally, a combination of bioinformatics methods is used for identifying candidate genes of interest for more detailed investigation. The biological significance of changes in expression of these genes should be validated in independent studies using Northern blot hybridization or quantitative RT-PCR to confirm altered expression of selected transcripts. The ultimate validation of microarray data rests on demonstrating altered expression of functions of proteins involved in biological processes that are suspected to contribute to the specific biological outcomes in the system subjected to gene expression profiling. An example of an informative microarray experiment conducted under MIAME specifications is described later in this work for analysis of mouse astrocytes exposed to HIV-1.

Functional genomics in the analysis of neurodegenerative diseases, including HAD

Functional genomics as a method of study of disease states has been applied with great success to identification of complex molecular pathways in carcinogenesis and more precise classification, detection, and prognosis of some cancers (Golub et al. 1999; Khan et al. 2001; Martin et al. 2001; Ramaswamy et al. 2003; Parmigiani et al. 2004). Application of functional genomics to diseases of the human nervous system has been slower, in part because these diseases involve multiple cell types rather than clonal expansion of a single-cell type, and the relevant tissues are generally not accessible during the lifetime of the patient (Glanzer et al. 2004). Nonetheless, large-scale gene expression profiling of tissues obtained at autopsy has revealed potential pathogenic pathways in Alzheimer's disease (Blalock et al. 2004; Lukiw 2004), chronic schizophrenia (Hakak et al. 2001), multiple sclerosis (Lock et al. 2002), and viral encephalitis (Gebicke-Haerter 2005).

Functional genomics analyses of brain tissues from individuals with HIV CNS disease have begun to appear (Gelman et al. 2004; Masliah et al. 2004; Shapshak et al. 2004). Masliah et al. (2004) and Gelman et al. (2004) found a number of significant gene expression changes in the cortex of patients who died with HIV encephalitis (HIVE). Follow-up studies established a correlation between use of methamphetamine and up-regulation of interferon genes in patients who died with HIVE (Everall et al. 2005) and identified somatostatin as a potential marker of MDD in HIVE patients (Everall et al. 2006). Experimental SIV infections conducted in macaques identified several functional clusters that were up-regulated in encephalitic brain, including several histocompatibility antigens involved in antigen presentation and interferon-related genes (Roberts et al. 2003, 2004). The human and macaque microarray studies for which sufficient information was available for comparative analysis with studies in culture are listed in Table 1.

It is clear that gene expression profiling of unfractionated brain tissue informs about the relative "average" RNA levels present in the tissue extract, regardless of any differences in expression in the individual cell types. Still, one can make predictions based on a relative abundance of cell types in the brain and changes in expression of cell-specific transcripts. With astrocytes constituting 50–60% of brain cell volume (Schubert 1984), it can be postulated that many of the differentially expressed genes in molecular profiles obtained from brain tissues of patients with HIV-1 neurocognitive disease represent physiological changes in astrocytes in vivo. It follows that comparisons of gene expression profiles of HAD brains with those from astrocytes subjected to pathogenic stimuli like HIV-1 in vitro may shed light on the role of astrocytes in HAD.

Gene expression profiling of human astrocytes challenged with HIV-1 or HIV-1 proteins

First attempts dedicated to identifying transcriptional responses of human astrocytes to HIV-1 used rapid subtraction hybridization (RaSH; Su et al. 2002, 2003). Fifteen transcripts were identified as being induced by the virus or viral gp120 envelope protein and termed astrocyte elevated genes (AEG); 10 genes were suppressed after HIV-1 infection or gp120 exposure and termed astrocyte suppressed genes or ASG (Su et al. 2002, 2003). Both AEG and ASG displayed temporal patterns of altered expression after HIV-1 infection of gp120 exposure; for example, expression of AEG-3 was highest 6 h after HIV-1 or gp120 exposure and declined thereafter, while expression of AEG-1 increased over time and peaked 7 days after the HIV-1 or gp120 stimulus (Su et al. 2003). These results demonstrated that human primary astrocytes respond to HIV-1 with extensive and temporal changes in cellular gene expression. The exposure to exogenous gp120 closely paralleled the effects of HIV-1 (Su et al. 2002, 2003), confirming previous observations indicating that gp120 binding itself, independent of infection, can exert profound global effects on astrocyte physiology (Benos et al. 1994; Wang et al. 2003).

RaSH and other subtraction hybridization methods are not high-flux procedures, and they have been designed primarily for discovery and cloning of novel differentially expressed genes (Boukerche et al. 2007). One of the genes discovered by RaSH in HIV-1-treated human astrocytes, AEG-1, was subsequently cloned and characterized as a mild tumor promoter and a factor controlling the expression of the astrocytic glutamate transporter EAAT2 (Kang et al. 2005).

Several studies undertook comprehensive gene expression profiling of astrocytes exposed to HIV-1 or viral proteins using high-flux microarray platforms for parallel detection of multiple differentially expressed genes (Galey et al. 2003; Kim et al. 2004; Kramer-Hämmerle et al. 2005). These reports are listed in Table 1. The table also includes the microarray analysis of primary mouse astrocytes exposed to HIV-1 described in this work (next section and Table 2 and Fig. 1). Finally, for comparison with astrocyte studies in vitro, Table 1 lists gene expression profiling studies with brain tissues from patients with HAD and SIV or SHIV-infected macaques. For all studies listed, we included information about the source of material used for microarray analysis, the microarray method used, the stimulus used in vitro or brain pathology for brain arrays, the total number of significantly changed (dysregulated) genes, and the main conclusions of the work. Each study in vitro found that HIV-1 or its proteins had profound effects upon astrocyte gene expression; it follows that in the HIV-1-infected brain, astrocytes subjected to HIV-1 exposure and infection are likely to suffer transcriptional changes that may contribute to the process of HAD.

All the studies we discuss (Table 1) compare cellular RNA expression in a ground state to an infected or treated state. As discussed in previous sections, the methods chosen to obtain and interpret the findings are diverse. Several different microarray platforms, from spotted microarrays using two-channel detection (i.e., Cy3–Cy5 microarrays) to oligonucleotide microarray using one-channel detection (i.e., Affymetrix GeneChip), were employed. Data analysis and statistical methods also differed among analysis platforms and among biological materials, and in some cases, statistical method was not listed. Assignment of biological significance to the changes in gene expression observed also varied from study to study. To some extent, the acceptable cut-off of gene fold changes (FCs) and significance analysis depend on the uniformity of the material used and robustness of results. For example, there is less variability in gene expression patterns among replicate samples of cultured cells, especially cell clones, than among brain tissue samples from different

individuals, even if they present with similar pathology. The number of replicas and statistical analysis approaches need to adjust to the nature of the biological source of data. Finally, most of the studies listed in Table 1 provided some functional classification of significantly regulated transcripts. Since there are a large number of gene ontology analysis programs and one gene can participate in several different pathways (Pavlidis et al. 2004; Werner 2008), the functional classification of the genes listed can differ from one study to another. Because not all the studies listed provided raw data in print or online, our comparison was limited to the final sets of significantly changed genes presented in each publication. Some studies could not be included in Table 1 because of insufficient information about the dataset discussed. Overall, with the caveats listed in the previous section, many commonalities regarding HIV-1 associated pathogenic changes in gene expression in astrocytes can be discerned.

The most general observation from the three studies with human astrocytes listed in Table 1 is that the cells respond profoundly to HIV-1 or HIV-1 proteins with broad and significant changes in cellular gene expression. The extent of the changes detected depended on the gene chip platform used and viral stimulus employed. Galey et al. analyzed gene expression pattern of human fetal astrocytes exposed to HIV-1 or to gp120 using lineage-specific immune- or neuro-microarrays containing 1,153 transcripts each (Galey et al. 2003; Table 1). These analytical platforms query a limited number of transcripts but address the expression of genes that are either functionally coordinated or act within a broad biological category. Of the target 2,306 genes in both arrays, 295 transcripts in total (12%) were found modulated by HIV-1 and/or gp120. Most of the transcripts were altered in expression either by HIV-1 or by gp120, with only 17 genes regulated similarly by both agents. Expression of 10 genes was altered by both stimuli, but in opposite directions. Chemokine and cytokine induction occurred predominantly in HIV-1-infected cells and not gp120 exposed cells (Galey et al. 2003). This report shows that HIV-1 induces a number of well-defined immune response genes in astrocytes as well as alters expression of a broader category of genes involved in nervous system functions. The differential signaling by HIV-1 and gp120 in astrocytes in this work indicates that gp120 is not the only component of intact virus that signals to astrocytes in long-term exposure. Indeed, since astrocytes in this study were tested 3 days after HIV-1 infection or gp120 exposure (Galey et al. 2003), the gene changes in HIV-1-infected cells likely reflected the activity of intracellular Tat or Nef proteins.

A more comprehensive gene expression profiling study in human fetal astrocytes challenged with HIV-1 employed the Affymetrix human GeneChip U133 A/B, which detected 44,000 transcripts (Kim et al. 2004). Distinct from a natural exposure of astrocytes to HIV-1, when infection occurs in a minority of cells (Li et al. 2007), this study investigated the pattern of cellular gene expression under the condition of widespread expression of HIV-1 throughout the astrocyte population (Canki et al. 2001). Human fetal astrocytes were infected with HIV-1 pseudotyped with the envelope glycoprotein G of vesicular stomatitis virus (VSV-G) permitting virus expression in 60–80% of human fetal astrocytes and subjected to microarray analysis at the peak of infection (Kim et al. 2004). Expression of over 730 genes was significantly altered. The most significantly up-regulated genes in these analysis included interferon-related genes as STAT1, IFNα21, IRF7, OAS1, IFIT1, MX1, and other genes involved in intercellular contacts, cell adhesion, intracellular signaling, transcriptional regulation, physiological processes, and others. Also, the complement C3 component was significantly up-regulated. Down-regulated genes cluster the most significant in cell cycle, DNA replication, and cell proliferation pathways (Kim et al. 2004).

In the third astrocyte study listed in Table 1, Kramer-Hämmerle analyzed the transcriptome of human astrocytes stably expressing HIV-1 Nef. Nef is an early viral protein that increases the pathogenicity of the virus (Greenway et al. 2003). Nef protein expression was also found

in astrocytes in brains of HIV-1-infected children with encephalitis (Saito et al. 1994). The most represented up-regulated pathways by native Nef protein in contrast to myristoylation-defective Nef-variant were implicated in small GTPase signaling, apoptosis, and lipid metabolism. Many of the genes identified were connected to the JAK/STAT and the MAPK signaling pathways.

Gene expression profile in murine astrocytes exposed to HIV-1

Although rodent cells lack HIV-1 receptors and are therefore not susceptible to native HIV-1 infection, cultured rodent astrocytes, neurons, and animals themselves have proven to be useful as model systems for studying neuropathogenic effects of HIV-1 virions or individual viral proteins (Benos et al. 1994; Toggas et al. 1994; Sui et al. 2006; Alirezaei et al. 2007; El-Hage et al. 2008; Wang et al. 2008). Here, we present for the first time global gene expression profile of primary mouse astrocytes exposed to intact HIV-1 in culture. Day2 neonatal mouse astrocytes in a third passage in culture were exposed in triplicates to cell-free HIV-1/NL4-3 at m.o.i. of 1 and cultured for 24 h. As control, cells were treated in triplicates with a mock virus concentrate at the same dilution (vol/vol) as the wild-type virus. As an example of large-scale gene expression analysis, we shall provide some details of the method; full description of the procedure is in Kim et al. (2004). Total RNA was prepared from cell cultures using the RNAeasy total RNA extraction kit (Qiagen, CA, USA). RNA quality was assessed by electrophoresis and spectrophotometric analysis; 2μg of total RNA was used to generate a cDNA, and then 1μg of cDNA product was used in an in vitro transcription reaction that contained biotinylated UTP and CTP. Twenty micrograms of full-length cRNA was fragmented and was subjected to gene expression analysis on the Affymetrix U74 mouse array chip. Scanner results were analyzed using ArrayAssist software (Stratagene; http://www.stratagene.com). Probe level analysis was performed using RMA (Robust Micro Array) algorithm. After verification of data quality by Affymetrix internal controls and signal distribution analysis as described in Stratagene Array Assist Protocol, data were transformed using variance stabilization and logarithm transformation with a base of 2. Values were normalized by mean intensities of all chip samples. Means of normalized expression values were calculated for triplicates of individual systems, and these values were used to calculate FC in the transcript. Genes showing FC values above 2 or below −2 and unpaired *t* test *p* values of <0.05 were defined as significantly changed. By these cut-off criteria, we identified 124 up-regulated and 87 down-regulated transcripts. Table 2 lists the majority of the significantly changed genes in this experiment grouped into functional categories using gene ontology tools available at the NetAffx™ Analysis Center (http://www.affymetrix.com/analysis/index.affx). The up-regulated pathways included immune and inflammatory responses and signal transduction whereas cell adhesion, signal transduction and protein modification were down-regulated (Table 2). A heatmap representing expression of up-regulated inflammatory cytokines in this system is showed in Fig. 1.

These results indicate that exposure of mouse astrocytes to HIV-1 in vitro causes broad and significant changes in the gene expression profile of these cells and that many of the changes resemble those observed by microarray analyses in human astrocytes exposed to HIV-1 or gp120 (Galey et al. 2003; Kim et al. 2004). It should be noted that such transcriptional profiles, although comprehensive in terms of the number of cellular transcripts analyzed, do not provide full picture of the biological changes that may occur in stimulus-responding cells. Many cellular functions are subject to post-transcriptional regulation, for example by alternative splicing of mRNA (Maragakis et al. 2004), translational regulation of protein expression by untranslated regions of mRNA (Kim et al. 2003; Irier et al. 2009), changes in protein stability (Arancibio-Cárcamo and Kittler 2009; Park et al. 2009), or post-translational protein modifications through acetylation or phosphorylation (Maestre et al.

2008; MacDonald and Roskams 2009), and such responses would not be detected by microarray analysis. Furthermore, the large amount of gene expression data generated in these experiments necessitates an arbitrary definition, based on a statistical paradigm chosen, of what constitutes a significant change in gene expression. Customarily, gene changes are considered significant if fold changes are above 2.0 and unpaired *t* test *p* values are <0.05 (Cui and Churchill 2003; Jafari and Azuaje 2006; De Muth 2009). This may result in missing biologically meaningful processes if the genes involved changed less than 2-fold (or more than −2.0 fold). Conversely, with a *p* value of 0.05, 5% of the observed changes may be due to chance. The *p* values are a statistical measure of reproducibility of replicate systems in an experiment or reproducibility between replicate experiments (De Muth 2009), and thus, lower *p* values instill better confidence in an array experiment. In the mouse astrocyte microarray study shown in this work, the majority of test results were lower than $p=0.01$ with some reaching $p=10^{-5}$ to 10^{-7} (Table 2), indicating that few gene changes observed here were due to chance. However, regardless of the statistical treatment and the stringency of microarray results, the lists of significantly altered genes placed within functional pathways such as shown in Table 2 and Fig. 1 are most valuable as a molecular map for posing hypotheses that integrate knowledge from many diverse experiments with a given system.

Comparison of gene profiling studies in astrocytes exposed to HIV-1 or HIV-1 proteins

Despite the different microarrays approaches and systems used, many specific changes in gene expression were found in two or more studies (Table 3). The highest correlation was observed between the Galey and Kim studies. Both used primary human fetal astrocytes. Galey et al. exposed astrocytes to either gp120 or HIV-1, whereas Kim et al. uses HIV-1 pseudotyped with VSV-protein G to increase the frequency of infection. Four gene changes in Kim's work correlated with Galey's HIV system and eight with Galey's gp120 (Table 3). The fact that there is no greater correlation in gene expression patterns in both models can be explained in part by different microarray approaches used or by differences in virus strains. Two different ways to alter astrocytic function by HIV-1 were previously described (Wang et al. 2004): Although only a small fraction of astrocytes are infected in vivo (Nuovo et al. 1994; Takahashi et al. 1996; Trillo-Pazos et al. 2003) and in vitro (Tornatore et al. 1991; Nath et al. 1995), the infection is not cytolytic, allowing cells to survive during perturbation by virus. Independent of productive infection, astrocytes respond to binding HIV-1 or envelope protein gp120 with functional changes consistent with changes in gene expression (Wang et al. 2004). Those two mechanisms to alter astrocyte function by HIV-1 may explain in part the differences observed in the two studies of HIV-1 exposure to astrocytes in culture that were compared.

The approach of Kramer-Hämmerle et al. using an astrocyma cell line expressing Nef protein shows many genes in common with Galey and Kim approaches. Common genes between HIV-1 and Nef expressing cells likely represent the expression changes induced by Nef protein after astrocyte infection by HIV-1. In addition, CTNNA1, ERCC2, and SREBF1 were found altered in astrocytes exposed to gp120 alone or expressing Nef (Table 3).

In general, genes identified as up-regulated by more than one analytical approach discussed here include IFN-related genes (STAT1 and IFITM1), cell adhesion molecules (CHL1, ICAM1, ITGAM, ITGB1, and CTNNA1), and transcription factors (STAT1 and SREBF1; Table 3). The DNA repair gene ERCC2 that can play a role in degradation of retroviral cDNA (Yoder et al. 2006) and CUL4A gene were also induced. Expression of this gene is necessary for HIV-1 Vpr-induced cell cycle arrest (Tan et al. 2007), and its induction is consistent with the down-regulation of cell-cycle-related genes observed in other studies

(Kim et al. 2004; Masliah et al. 2004). Down-regulated genes identified by Galey and Kim included the transcription factor TRIP13, the gene FPR1 that is implicated in some inflammatory processes and chemotaxis, IGFBP3 involved in cell proliferation and apoptotic processes, and the metabolic genes UGP2 and APEH.

We also compared cellular transcripts altered in HIV-1-exposed mouse astrocytes to those obtained in gene profiling studies in HIV-1-infected human astrocytes (Table 3). Surprisingly, transcriptomes from HIV-1-treated mouse and human astrocytes showed many commonalities despite the different species and microarray technologies used (Table 3). We found correlation for several genes in mouse astrocytes and Galey and Kim studies. Many of these genes are implicated in immune responses (IL-10, CD44, CXCL1, and CXCL2). Some of the significantly changed genes common to mouse and human astrocytes are of interest in the context of the neuropathology observed in HIV-associated dementia. For instance, the gene regulator of G-protein signaling 4 (RGS4) modulates signal transduction through several neurotransmitter receptor systems and has been reported as being implicated in many neurological disorders like schizophrenia, bipolar disorder, Huntington disease, or behavioral abnormalities (Ding and Hegde 2008; McOmish et al. 2008; Runne et al. 2008). Down-regulated genes FLT1 and PTPRO may be implicated in neurogenesis and axon guidance respectively and may participate in memory/learning processes (Cao et al. 2004; Chen and Bixby 2005; Zhang et al. 2005; Krum et al. 2008). This analysis confirms at a functional genomics level that mouse astrocytes, similar to mouse neurons and the animals themselves, are suitable models for investigation of many aspects of HIV-1 pathogenesis.

Comparison with infected brain tissues

One of the models used to study HIV-associated dementia has been the infection with SIV in rhesus macaques. This model reproduces many aspects of HIV brain disease in people, including CNS infection and dysfunction (Roberts et al. 2003) with cognitive and motor problems and the development of SIV encephalitis. This experimental infection has also been analyzed for changes in cellular gene expression associated with immunodeficiency virus infection in the brain. Sui et al. (2003) and Buch et al. (2004) used SHIV (virus bearing the envelope of HIV-1 on the background of SIV) as a model of neurological disease. Using microarrays, they analyzed the gene changes induced in the brains of SHIV-infected macaques with encephalitis when compared to those without encephalitis. Another comparison was made by Roberts et al. (Roberts et al. 2003, 2004) using SIV-infected macaque brains to study SIVE and acute SIV infection. They performed microarray analysis in the frontal lobe of the infected macaques with acute SIV infection, different time points after infection, and SIVE and compared their expression levels with the uninfected control cases. Many of the genes show an increased expression during the acute phase of infection, long-term infection, and SIVE (IFN- and IL-6-related genes). Both approaches show an up-regulation of genes implicated in the inflammatory response, including promoting monocyte infiltration, differentiation, and activation in the brain. Focusing upon cytokine expression, Stephens et al. used cytokine microarrays and identified eight genes induced in brains of macaques infected with SHIV compared to uninfected controls among them IFI6, PTN, and IL-6 that were also identified in other studies (Table 3).

A study comparing brains from HIV-1-infected patients with and without HIVE conducted by Masliah et al. 2004 show that interferon-related genes and neuro-inflammatory responses were up-regulated in patients with HIVE. These data are consistent with the results observed in SIVE and in cultured astrocytes. Down-regulated pathways included synaptic plasticity and transmission, cell cycle, and signaling molecules. Gelman et al. (2004) focused upon specific gene families and identified many dysregulated ionic conductance carriers in HAD or mild cognitive motor disorder compared with brains from uninfected people. Such

neuronal channelopathies can alter membrane excitation and are consistent with some of the features of HAD. A study focusing on the expression of toll-like receptors (TLR)-related genes conducted by Salaria et al. (2007) found a correlation between expression levels of TLR-related genes and neuropathological parameters indicators of neurodegeneration.

Looking across the studies cited and comparing the individual genes dysregulated in human astrocytes and in macaque and human brain, one finds a significant number of genes that are similarly regulated (Table 3). Those genes are mostly implicated in immune response, including interferon-related genes (STAT1, IFIT1, IFIT4, IFITM1, IFI6, IRF1, MX1, and ISG15), other immune-related genes (HLADRB1, LGALS3BP, and BST2), and other genes implicated in brain function (DTNA, GFAP, MDK, and ZIC1). Two genes were down-regulated in macaque brain and human astrocytes (IGFBP3 and ERBB3), and only one gene was found down-regulated in human brain and the astrocyte model, FXYD2, a gene probably implicated in glutamate uptake by EAAT1 in astrocytes (Gegelashvili et al. 2007). Many other genes up-regulated in mouse astrocytes were also up-regulated in brain tissue of macaque SIV or SHIV model of encephalitis. They include immune response genes (IL1A, CXCL10, CD44, and IL-6), oxidative stress genes (SOD2), and others as CHI3L1. Alteration in CHI3L1 expression was associated with schizophrenia (Chung et al. 2003; Table 3).

Together, these results suggest that HIV-1-exposed and/or infected astrocytes may participate in the inflammatory responses observed in patients with HIVE. Because many of the transcriptional changes observed in HIV-1-exposed astrocytes in vitro can also be found in other cells involved in HIV-1 brain disease, particularly microglial cells and macrophages (Lipton and Gendelman 1995; Kolson and Pomerantz 1996), it is important to determine whether any of these molecular markers are also (or exclusively) altered in astrocytes in vivo. Thus far, besides routine detection of GFAP as a marker of gliosis (Budka 2005), there are only a few reports on expression of other cellular markers by astrocytes in brains from HIV-1-infected patients. Using in situ hybridization, Nuovo and Alfieri (1996) showed elevated chemokine MIP-1α and MIP-1β RNA in astrocytes in brain tissues of an AIDS patient (Nuovo and Alfieri 1996). More recently, Torres-Muñoz and colleagues identified elevated glial clusterin in astrocytes in tissue sections from brains of patients with HIV-1 dementia and suggested that clusterin may serve as a marker of neuroprotective function of astrocytes independent of GFAP expression (Torres-Muñoz et al. 2001b). Interestingly, MIP-1α RNA was significantly up-regulated (FC of 2.22; $p=6\times10^{-6}$) in HIV-1-exposed astrocytes in vitro (Table 2). Clusterin gene was also up-regulated in our astrocyte study (FC of 1.30 and $p=0.027$) but was not included in Table 2 because of our cut-off criteria. Other factors in the brain besides HIV-1, such as cytokines or viral proteins secreted by infected/activated microglia, could trigger induction of MIP-1α and clusterin in astrocytes in the cited studies. Nevertheless, analysis of glial transcriptome profiles in vitro together with rigorous correlative studies in patients' tissues or animal models can be useful for elucidating the role of astrocytes in HIV-associated dementia.

Other studies in astrocytes using functional genomics with potential implications for HAD

Astrocytes actively respond to a wide array of environmental stimuli and can participate in local innate immune responses in the brain. It has been demonstrated that astrocytes express receptors of the TLR family and that following activation, astrocytes secrete chemokines and cytokines that can not only mediate immune responses but also contribute to neuroinflammation (for review, see Farina et al. 2007). Rivieccio et al. (2005, 2006) employed microarray analysis to determine global gene expression profiles of human fetal astrocytes stimulated with IL-1β or with polyriboinosinic polyribocytidylic acid (pIC), a

TLR3 ligand analog. IL-1β and pIC induced extensive gene transcription changes in astrocytes, including up-regulation of IFN-stimulated genes, chemokines, and other genes participating in inflammation. pIC was more effective than IL-1β in activating antiviral response genes. Many of the genes induced in astrocytes by IL-1β and/or pIC, including the antiviral response genes MX1, IFIT1, OAS1, IRF7, or the chemokine CXCL1, were also induced in HIV-1-infected astrocytes (Kim et al. 2004; Table 1). It is noteworthy that the chemokines CXCL1 and CXCL10 and the cytokine IL-6 were also up-regulated in mouse astrocytes exposed to HIV-1 (Table 2). These results indicate that HIV-1 can be considered a mediator of inflammatory responses in astrocytes on par with natural inflammatory stimuli such as IL-1β. In another example, Meeuwsen et al. (2003) compared gene expression profiles of astrocytes exposed to proinflammatory cytokines TNF-α and IL-1β. Up-regulated genes included chemokines, growth factors, and receptors. Some of the genes induced in astrocytes by TNF-α or IL-1β described in this work were up-regulated by HIV-1 or SIV in vivo (Table 1). Other microarray analyses have shown that exposure of astrocytes in culture to different viruses (Cai et al. 2003; Radhakrishnan et al. 2003; Aravalli et al. 2006; Rubio and Sanz-Rodriguez 2007), conditions of hypoxia (Mense et al. 2006), and environmental toxins (Everall et al. 2005; Mense et al. 2006; Sengupta et al. 2007) can all significantly alter gene expression profiles of these cells. While the results of such studies cannot be directly extrapolated to pathogenic situations in HIV-1-infected brains, they clearly indicate that astrocytes are highly reactive cells and that their physiological functions can be profoundly altered by contact with a wide variety of natural and pathogenic stimuli, including HIV-1.

Conclusions

We have attempted to integrate findings from several experimental platforms using microarray analysis that reveal HIV-1 effects on gene expression in astrocytes. HIV-1 or HIV-1 proteins gp120, Nef, or Tat can induce innate immune effectors, interferon-stimulated genes, and other gene families in human astrocytes. Many of the individual genes with altered expression were found in common in more than one analysis, pointing to their potential significance in the HIV-1 pathogenic process. Similar gene expression changes were found in HIV-1-exposed mouse astrocytes in vitro, underscoring the usefulness of the mouse for study of HIV-1 pathogenesis. At another level of comparison, we also found commonalities in the changes induced by HIV-1 in culture with those observed in the brain of SIV-infected macaques or HIV-1-infected patients. Many up-regulated genes were found both in effectively pure astrocyte cultures and in mixed-cell tissue extracts from infected brains. This strongly suggests that changes in gene expression of astrocytes are a major component of the overall molecular profile of disease in the brains of HIV-1-infected people. It further suggests that exposure of astrocytes to HIV-1 in culture can be a useful tool for investigating the molecular and functional changes, leading to the development of HIV-associated dementia.

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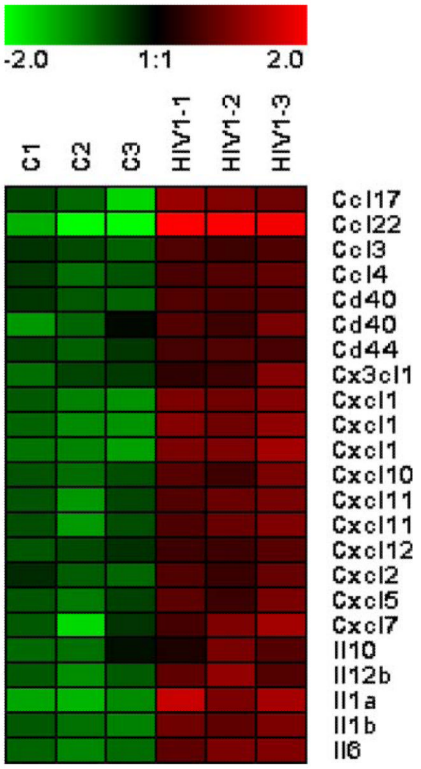

Fig. 1.

Heatmap representing the signal values of chemokines and cytokines up-regulated in mouse astrocytes exposed to HIV-1. *Red color* represents up-regulation, and *green color* represents down-regulation. Uninfected controls are represented by C1, C2, and C3 and HIV-1-exposed mouse astrocytes by HIV1-1, HIV1-2, and HIV1-3. The selected chemokines and cytokines in the figure were significant with a *t* test *p* value of <0.05 and a fold change >2. The figure was generated using the values obtained from the ArrayAssist 5.5.1. software (Stratagene). Briefly, the RMA data values were calculated and transformed by variance stabilization, logarithmic scale, and baseline transformation. The final values were represented in a heatmap using Genesis software (http://genome.tugraz.at/)

Table 1

Gene profiling studies in HIV-1-infected astrocytes and HIV-1- or SIV-infected brains discussed in this review

Citation	Cells	Microarray	Virus stimulus	Analysis	Genes regulated/ total	Conclusions
Astrocytes						
Galey et al. 2003	Primary human astrocytes	NIA immuno and neuroarray	HIV-gp120	HIV or gp120 vs non-HIV	295/ 2,306	Differential effect of HIV-1 and gp120 in astrocytes. Gp120 has more profound effect but chemokine and cytokine induction occurs predominantly by HIV infection
Kim et al. 2004	Primary human astrocytes	Affymetrix U133 A/B	VSV-HIV	VSV-HIV vs non-VSV-HIV	734/ >44,000	Up-regulation of IFN antiviral responses, intercellular contacts, cell adhesion, and signaling. Down-regulation of cell cycle, DNA replication, and cell proliferation
Kramer-Hämmerle et al. 2005	Astrocytoma—Nef expressing cells	BD Bioscience Clontech	Nef	Native Nef vs non-myristoylated Nef	400/ 11,835	Up-regulation of small GTPase signaling, regulation of apoptosis, lipid metabolism. JAK/STAT and MAPK signaling pathways
Borjabad and Volsky 2009 (present work)	Primary mouse astrocytes	Affymetrix U74v2	HIV	HIV vs non-HIV	211/ 36,000	Up-regulation of immune response, inflammation, signal transduction, and chemotaxis. Down-regulation of signal transduction, development, and protein modification processes
Brain tissue						
Sui et al. 2003	Macaque—basal ganglia	Clontech chemokine and cytokine array	SHIV	SIVE vs non-SIVE	50/277	Changes in cytokine and its receptors expression during SHIV-encephalitis. Up-regulated genes participating in promoting monocyte infiltration, macrophage activation, and enhancement of virus replication. Down-regulation of neutrophic factors
Roberts et al. 2003	Macaque—frontal lobe	Affymetrix U95Av2	SIV	SIVE vs ni	98/ 12,625	Up-regulation in SIVE of genes implicated in monocyte entry to the brain, inflammation, IFN response, antigen presentation, production of neurotoxic effects, transcription factors, and others
Roberts et al. 2004	Macaque—frontal lobe	Affymetrix U95Av2	SIV	SIV vs ni	97/ 12,625	Up-regulation in acute SIV infection of genes involved in IFN and IL-6 pathways. Many of these genes also up-regulated in long-term infection and SIVE
Stephens et al. 2006	Macaque—cortical brain	Clontech Cytokine array	SHIV	SHIV vs ni	8	Up-regulated genes, including Cripto-1 and genes implicated in inflammatory, neuroprotective, cognitive, and stress responses
Masliah et al. 2004	Human—frontal cortex	Affymetrix U95Av2	HIV	HIVE vs non-HIVE	133/ 12,625	Up-regulated pathways included neuroimmune and antiviral response, transcription, and signaling pathways. Down-regulated pathways included synaptic plasticity and transmission, cell cycle, signaling molecules, transcription factors, and cytoskeletal components

Table 2

Partial list of cellular significantly changed genes in mouse primary astrocytes exposed to HIV-1

Up-regulated genes				**Down-regulated genes**			
Genet title	**Gene symbol**	**FC**	***t* test**	**Gene title**	**Gene symbol**	**FC**	***t* test**
Chemokines				Signal transduction			
Chemokine (C–C motif) ligand 22	Ccl22	15.18	0.0002	Regulator of G-protein signaling 2	Rgs2	−4.69	0.0004
Chemokine (C–X–C motif) ligand 1	Cxcl1	4.49	0.0001	G-protein-coupled receptor 34	Gpr34	−3.20	0.0009
Chemokine (C–C motif) ligand 17	Ccl17	4.16	0.0042	RAS, guanyl releasing protein 3	Rasgrp3	−2.98	0.0063
Chemokine (C–X–C motif) ligand 7	Cxcl7	3.70	0.0142	Chimerin (chimaerin) 2	Chn2	−2.22	0.0000
Chemokine (C–X–C motif) ligand 11	Cxcl11	3.10	0.0020	Epstein–Barr-virus-induced gene 2	Ebi2	−2.15	0.0006
Chemokine (C–X–C motif) ligand 5	Cxcl5	2.71	0.0017	Purinergic receptor P2Y, G-protein-coupled 13	P2ry13	−2.06	0.0010
Chemokine (C–X–C motif) ligand 10	Cxcl10	2.67	0.0009	Amyloid beta (A4) precursor protein binding A1	Apba1	−2.05	0.0076
Chemokine (C–C motif) ligand 4	Ccl4	2.51	0.0006	Protein tyrosine phosphatase, receptor type, O	Ptpro	−2.02	0.0125
Chemokine (C–X–C motif) ligand 2	Cxcl2	2.39	0.0023	RAB31, member RAS oncogene family	Rab31	−2.01	0.0309
Chemokine (C–X3–C motif) ligand 1	Cx3cl1	2.38	0.0068	Cell adhesion			
Chemokine (C–C motif) ligand 3	Ccl3	2.22	0.0006	Osteomodulin	Omd	−3.46	0.0033
Chemokine (C–X–C motif) ligand 12	Cxcl12	2.15	0.0006	CD34 antigen	Cd34	−3.44	0.00001
Interleukines				Procollagen, type XIV, alpha 1	Col14a1	−3.22	0.0011
Interleukin 1 alpha	Il1a	5.95	0.0002	Prostaglandin-endoperoxide synthase 1	Ptgs1	−3.02	0.0001
Interleukin 6	Il6	3.52	0.0001	Nephronectin	Npnt	−2.67	0.0136
Interleukin 1 beta	Il1b	3.36	0.0001	Metastasis suppressor 1	Mtss1	−2.38	0.0086
Interleukin 12b	Il12b	3.24	0.0012	Phosphoglucomutase 5	Pgm5	−2.18	0.0117
Interleukin 10	Il10	2.36	0.0186	Neural cell adhesion molecule 1	Ncam1	−2.04	0.0319

Up-regulated genes				Down-regulated genes			
Genet title	**Gene symbol**	**FC**	***t* test**	**Gene title**	**Gene symbol**	**FC**	***t* test**
Inflammation				Sidekick homolog 2 (chicken)	Sdk2	−2.02	0.0010
Selectin, platelet	Selp	2.87	0.0006	Apoptosis			
Acyloxyacyl hydrolase	Aoah	2.86	0.0010	Insulin-like growth factor 1	Igf1	−2.88	0.00004
Selectin, platelet	Selp	2.56	0.0004	B-cell leukemia/lymphoma 2	Bcl2	−2.38	0.0079
Tumor necrosis factor receptor superfamily, member 1b	Tnfrsf1b	2.03	0.0001	Serum/glucocorticoid-regulated kinase	Sgk	−2.01	0.0001
Complement activation				Metabolism			
Complement factor B	Cfb	2.60	0.0016	Hydroxyprostaglandin dehydrogenase 15 (NAD)	Hpgd	−7.52	0.0001
Complement component 1, r subcomponent	C1r	2.02	0.0030	Phosphotriesterase related	Pter	−3.43	0.0005
Other immune response				C1q-like 3	C1ql3	−2.26	0.0013
Fc receptor, IgG, low affinity IIb	Fcgr2b	4.26	0.0003	Arachidonate 5-lipoxygenase activating protein	Alox5ap	−2.11	0.0001
Gene model 1960, (NCBI)	Gm1960	3.75	0.0004	Phosphorylase kinase beta	Phkb	−2.04	0.0001
C-type lectin domain family 4, member e	Clec4e	2.69	0.0017	Phospholipase C-like 1	Plcl1	−2.01	0.0432
Toll-like receptor 1	Tlr1	2.65	0.0001	Development			
CD40 antigen	Cd40	2.62	0.0176	Exostoses (multiple) 1	Ext1	−2.90	0.0072
2′–5′ oligoadenylate synthetase-like 1	Oasl1	2.61	0.0046	Sloan–Kettering viral oncogene homolog	Ski	−2.65	0.0016
Histocompatibility 2, Q region locus 8	H2-Q8	2.49	0.0004	Angiogenin, ribonuclease A family, member 1	Ang1	−2.22	0.0120
Colony-stimulating factor 2 (granulocyte–macrophage)	Csf2	2.42	0.0333	Motile sperm domain containing 3	Mospd3	−2.13	0.0485
Immunoresponsive gene 1	Irg1	2.34	0.0014	Ribonuclease, RNase A family 4	Rnase4	−2.04	0.0004
T-cell specific GTPase	Tgtp	2.28	0.0008	Protein biosystesis and modification			
Repeats 1	LOC667373	2.28	0.0002	Transforming growth factor, beta receptor I	Tgfbr1	−2.95	0.0236
Histocompatibility 2, K1, K region	H2-K1	2.03	0.0014	FMS-like tyrosine kinase 1	Flt1	−2.70	0.0128
Macrophage activation 2 like	Mpa2l	2.03	0.0009	Zinc and ring finger 4	Znrf4	−2.58	0.0113
Apoptosis				Ribosomal protein S13	Rps13	−2.48	0.0147
Receptor (TNFRSF)-	Ripk2	3.27	0.0012	E3 ubiquitin protein ligase,	Edd1	−2.46	0.0115

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Up-regulated genes				Down-regulated genes			
Genet title	**Gene symbol**	**FC**	***t* test**	**Gene title**	**Gene symbol**	**FC**	***t* test**
interacting serine–threonine kinase 2				HECT domain containing, 1			
Stam-binding protein	Stambp	2.15	0.0090	Chymotrypsin like	Ctrl	−2.17	0.0108
Caspase recruitment domain 4	Card4	2.03	0.0009	SUMO/sentrin specific peptidase 6	Senp6	−2.10	0.0080
Tumor necrosis factor, alpha-induced protein 3	Tnfaip3	2.01	0.0017	Protein kinase C, alpha	Prkca	−2.06	0.0362
Ion transport				Immune response			
Cytochrome P450, family 7, subfamily b, polypeptide 1	Cyp7b1	2.42	0.00003	Complement component factor h	Cfh	−2.89	0.0332
Potassium voltage-gated channel	Kcnf1	2.29	0.0013	Histocompatibility 2, class II, locus Mb1	H2-DMb1	−2.26	0.0113
Thioredoxin-like 1	Txnl1	2.11	0.0114	Defensin beta 1	Defb1	−2.03	0.0091
Polycystic kidney disease 1 like 3	Pkd1l3	2.04	0.0346	Ion transport			
Signal transduction				Solute carrier anion transporter family, member 2b1	Slco2b1	−2.67	0.0105
Phosphodiesterase 2A, cGMP-stimulated	Pde2a	3.36	0.0030	Potassium channel, subfamily J, member 16	Kcnj16	−2.17	0.0082
Thrombospondin type 1 motif, 4	Adamts4	3.33	0.0005	Solute carrier family 4 (anion exchanger), member 4	Slc4a4	−2.12	0.001
Membrane-spanning 4 domains	Ms4a4d	2.87	0.0006	Myelin basic protein expression factor 2, repressor	Myef2	−2.02	0.0249
Regulator of G-protein signaling 4	Rgs4	2.60	0.0053	Transcription			
Like sequence 4	Emr4	2.52	0.0003	Myeloid ecotropic viral integration site-related gene 1	Mrg1	−2.36	0.0204
Prostaglandin I receptor (IP)	Ptgir	2.34	0.0055	Cyclin L2	Ccnl2	−2.02	0.0294
Spi-B transcription factor (Spi-1/PU.1 related)	Spib	2.27	0.0322	Cell cycle			
BMP-binding endothelial regulator	Bmper	2.19	0.0015	SMC4 structural maintenance of chromosomes 4-like 1	Smc4l1	−2.15	0.0003
Adenosine A2b receptor	Adora2b	2.15	0.0324	Establishment of cohesion 1 homolog 2 (*S. cerevisiae*)	Esco2	−2.06	0.0301
Rho family GTPase 1	Rnd1	2.09	0.0000001				
Kinase suppressor of ras 1	Ksr1	2.08	0.0105				

Up-regulated genes				Down-regulated genes			
Genet title	**Gene symbol**	**FC**	***t* test**	**Gene title**	**Gene symbol**	**FC**	***t* test**
Somatostatin receptor 3	Sstr3	2.07	0.0291				
Transglutaminase 2, C polypeptide	Tgm2	2.02	0.0083				
Formyl peptide receptor 1	Fpr1	2.01	0.0002				
Regulation of transcription				Other functions			
NK2 transcription factor related, locus 3 (*Drosophila*)	Nkx2-3	2.39	0.0074	Insulin-like growth factor binding protein 5	Igfbp5	−3.11	0.0070
Forkhead-like 18 (*Drosophila*)	Fkhl18	2.32	0.0100	Cortactin-binding protein 2	Cttnbp2	−2.36	0.00002
Doublesex and mab-3-related transcription factor	Dmrta1	2.10	0.0103	RAD51-associated protein 1	Rad51ap1	−2.24	0.0022
Cells 2, p49/p100	Nfkb2	2.05	0.0020	HIV-1 Rev-binding protein like	Hrbl	−2.10	0.0017
Nuclear receptor interacting protein1	Nrip1	2.03	0.0057	RNA-binding region (RNP1, RRM) containing 3	Rnpc3	−2.07	0.0123
Cells inhibitor, epsilon	Nfkbie	2.01	0.0019	Heat shock protein 90 kDa alpha, class A member 1	Hsp90aa1	−2.06	0.0024
Metabolism				Mannose receptor, C type 1	Mrc1	−2.02	0.0028
Vanin 3	Vnn3	3.59	0.0001	WD repeat domain 1	Wdr1	−2.01	0.0100
Chitinase 3-like 1	Chi3l1	3.20	0.00003	Not classified function			
Prostaglandin E synthase	Ptges	2.89	0.0016	Tripartite motif protein 34	Trim34	−2.81	0.0195
Carbonic anhydrase 13	Car13	2.66	0.0038	CTD small phosphatase like 2	Ctdspl2	−2.59	0.0031
Prostaglandin-endoperoxide synthase 2	Ptgs2	2.47	0.0065	Membrane protein, palmitoylated 6	Mpp6	−2.52	0.0149
Urocanase domain containing 1	Uroc1	2.29	0.0050	DNA2 DNA replication helicase 2-like (yeast)	Dna2l	−2.52	0.0257
AMP deaminase 3	Ampd3	2.11	0.0012	Meningioma-expressed antigen 5 (hyaluronidase)	Mgea5	−2.46	0.0326
Matrix metallopeptidase 13	Mmp13	2.03	0.0214	Cytotoxic T lymphocyte-associated protein 2 beta	Ctla2b	−2.37	0.0011
Development				Leukocyte-associated Ig-like receptor 1	Lair1	−2.36	0.0025
Tumor necrosis factor, alpha-induced protein 2	Tnfaip2	3.10	0.0001	Glucosaminyl (*N*-acetyl) transferase 1, core 2	Gcnt1	−2.35	0.0004
(Semaphorin) 4A	Sema4a	2.88	0.0002	Cytoplasmic polyadenylation element binding protein 2	Cpeb2	−2.21	0.0161

Up-regulated genes				Down-regulated genes			
Genet title	**Gene symbol**	**FC**	***t* test**	**Gene title**	**Gene symbol**	**FC**	***t* test**
VGF nerve growth factor inducible	Vgf	2.43	0.0006	SERTA domain containing 4	Sertad4	−2.20	0.0011
Adrenomedullin	Adm	2.00	0.0381	RAB3A interacting protein (rabin3)-like 1	Rab3il1	−2.18	0.0026
Cell adhesion				Navigator 3	LOC676640	−2.16	0.0092
Thrombospondin 2	Thbs2	2.27	0.0189	Radial spokehead-like 3	Rshl3	−2.13	0.0030
CD44 antigen	Cd44	2.22	0.0004	Leucine zipper protein 2	Luzp2	−2.13	0.0262
Transport				Retinitis pigmentosa 9 homolog (human)	Rp9h	−2.10	0.0006
Solute carrier family 7, member 11	Slc7a11	4.25	0.0003	Nudix-type motif 10	Nudt10	−2.10	0.0053
Solute carrier family 1, member 2	Slc1a2	2.15	0.0053	Tetratricopeptide repeat domain 14	Ttc14	−2.10	0.0360
Syntaxin 11	Stx11	2.05	0.0071	Tudor domain containing 3	Tdrd3	−2.09	0.0232
Other functions				Coiled-coil domain containing 39	Ccdc39	−2.03	0.0090
Pentraxin-related gene	Ptx3	3.12	0.00002	DNA segment, Chr 3, ERATO Doi 300, expressed	D3Ertd300e	−2.02	0.0261
Tissue inhibitor of metalloproteinase 1	Timp1	3.10	0.00001	Cytotoxic T lymphocyte-associated protein 2 alpha	Ctla2a	−2.01	0.0033
Coagulation factor C homolog (*Limulus polyphemus*)	Coch	2.71	0.0221	Glypican 6	Gpc6	−2.00	0.0035
Superoxide dismutase 2, mitochondrial	Sod2	2.61	0.0002				
Actin-binding LIM protein 1	Ablim1	2.22	0.0405				
Protein C receptor, endothelial	Procr	2.09	0.00003				
Schlafen 1	Slfn1	2.04	0.0039				
Histone deacetylase 2	Hdac2	2.01	0.0060				
Not classified function							
leucine-rich repeat LGI family, member 2	Lgi2	5.31	0.0011				
Nuclear protein 1	Nupr1	3.40	0.0080				
Ladinin	Lad1	3.30	0.0012				
Z-DNA binding protein 1	Zbp1	2.95	0.0064				

Up-regulated genes				Down-regulated genes			
Genet title	**Gene symbol**	**FC**	***t* test**	**Gene title**	**Gene symbol**	**FC**	***t* test**
Cysteine-rich secretory protein LCCL	Crispld2	2.78	0.0073				
Serine (or cysteine) peptidase inhibitor	Serpinb5	2.78	0.0143				
Bassoon	Bsn	2.44	0.0140				
Additional sex combs like 2 (*Drosophila*)	Asxl2	2.42	0.0305				
Ventricular zone expressed PH domain homolog 1	Veph1	2.38	0.0048				
Purkinje cell protein 4	Pcp4	2.37	0.0068				
SLAM family member 8	Slamf8	2.27	0.0018				
Transmembrane protein 25	Tmem25	2.22	0.0148				
DENN/MADD domain containing 3	Dennd3	2.20	0.0293				
Zinc finger CCCH type containing 12A	Zc3h12a	2.13	0.0017				
Schlafen 4	Slfn4	2.13	0.0013				
Transmembrane protein 74	Tmem74	2.13	0.0282				
TNFAIP3 interacting protein 1	Tnip1	2.09	0.0011				
Ataxin 2	Atxn2	2.06	0.0304				
Glycophorin C	Gypc	2.04	0.0305				
ELOVL family member 7	Elovl7	2.03	0.00005				

Up- and down-regulated transcripts in primary mouse astrocytes exposed to HIV-1. Results are selected by the criteria: FC>2, *t* test *p* value <0.05. Genes are classified by biological pathways using NetAffx Analysis Center (Affymetrix)

Table 3

Comparison of significantly regulated genes in the different studies listed in Table 1

	Astrocytes					**Brain tissue**			
	Galey HIV	**Galey gp120**	**Kim**	**Kramer-Hämmerle**	**Borjabad and Volsky**	**Sui and Buch**	**Stephen**	**Roberts**	**Masliah**
Astrocytes	Galey HIV	STAT1 ITGAM **GFAP AKAP12 SLC9A3R1 PPP1CA BTF3 COL3A1 ALCAM MME PIP5KIC EFNB2 GRB2 GGCX RPS6 FPR1 CYC1**	CUL4A STAT1 **TRIP13 FPR1**	EPAC RRAD IGFBP3 ITGB1 PSMD2	RGS4 TIMP1 **PTPRO**	STAT1 SPARC	0	BAX VIM TIMP1 STAT1	TGFBR3
	Galey gp120		CHL1 ICAM1 STAT1 ERCC2 **IGFBP3 UGP2 FPR1 APEH**	CTNNA1 ERCC2 SREBF1	IL10 **FLT1**	STAT1 **IGFBP3**	0	STAT1 HLADRB1 CX3CR1 IFIT4	IFIT4 STAT1 GPR37
	Kim			IFITM1 SLC2A3 MGST1 SEMA4D ERCC2	SLC7A11 CD44 CHI3L1 CXCL1 CXCL2 **ESCO2 IGFBP5 NCAM1 RAD51AP1**	IRF1 ZIC1 CD44 STAT1 **ERBB3 IGFBP3**	IFI6	MX1 IFIT1 IFITM1 CHI3L1 ISG15 STAT1 BST2 LLGL1 DTNA	MX1 IFIT1 IFITM1 ISG15 BST2 STAT1 DTNA **FXYD2**
	Kramer-Hämmerle				ABLIM1 C1R LAD1 TNFAIP2	0	PTN	LGALS3BP GIP3 IFITM1 STAT3 MDK ITGB4 RAF1 IGFBP2	IFITM1 GFAP LGALS3BP
	Borjabad and Volsky					IL1A CXCL10 CD44 SOD2	IL6	CHI3L1 NUPR1 TGM2 TIMP1	0
Brain tissue	Sui and Buch						0	ISGF3G SPP1 FN1 GIP2 FCER1G STAT1	STAT1 EPHB1
	Sthepens							0	0
	Roberts								ISG15 IFITM1 IFIT1 IFIT4 IFI44 MX1 IGL@ BST2 HLA-A HLA-C HLA-F B2M STAT1 DTNA LGALS3BP
	Masliah								

Bold font indicates down-regulated genes