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Host and virus strain dependence in activation of human macrophages by human immunodeficiency virus type 1

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Human immunodeficiency virus type 1 (HIV-1)–associated neuropathogenesis occurs in a large minority of infected people. Presently, there are neither viral nor cellular markers that predict the development of brain disease during HIV-1 infection. This study was conducted to determine whether there exist systematic differences among human cell donors and virus strains for the activation of macrophage gene expression by HIV-1 that may contribute to neuropathogenesis. Four HIV-1, ADA and B-aL, which were isolated from peripheral tissues of acquired immunodeficiency syndrome (AIDS) patients, and DJV and YU-2, which were isolated from brains of patients with HIV-1–associated dementia, were compared for induction of expression of cellular genes associated with antiviral activity or inflammation in monocyte-derived macrophages from several donors. Virus replication and cytokine production were scored by enzyme-linked immunosorbent assay (ELISA) and cellular transcripts were measured by real-time polymerase chain reaction (PCR). ADA and B-aL productively infected cells from all donors tested and induced all cellular transcripts tested, illustrating a common response of macrophages to HIV-1 replication. In sharp contrast, the viruses associated with neuropathogenesis, DJV and YU-2, induced intense gene expression early after infection in cells from a subset of donors but DJV did not productively infect these cells. No such heterogeneity was observed in the responses of macrophages during high-level replication of any HIV-1 tested. The susceptibility to early activation by HIV-1 may reflect susceptibility to neuropathogenesis in AIDS. *Journal of NeuroVirology* (2007) **13,** 452–461.

Keywords: AIDS dementia; HIV heterogeneity; host polymorphism; macrophage

Introduction

Human immunodeficiency virus type 1 (HIV-1) infection was first recognized for its decimation of the human immune system (Gottlieb *et al*, 1981). Unlike immunopathogenesis, HIV-1 neuropathogenesis was observed only in a fraction of acquired immunodeficiency syndrome (AIDS) patients (reviewed in González-Scarano and Martín-García, 2005). Among the variables contributing to disease, virus residence in the central nervous system (CNS) is necessary but not sufficient for the development of HIV-1–associated dementia (HAD). In clinical studies the parameter best associated with HAD was the frequency of activated macrophages in the brain and not the number of HIV-1–infected cells (Brew *et al*, 1995; Glass *et al*, 1995). Paradoxically, effective antiretroviral therapy has greatly reduced the incidence of HAD, even when some drugs enter the CNS poorly (McArthur *et al*, 2003). There are cases of reversal of cognitive defects with antiretroviral therapy, clearly implicating virus replication as a driving force in brain disease (Gendelman *et al*, 1998). These

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findings tend to indicate that activation of cells of the macrophage lineage by HIV-1 infection or exposure in the periphery leads to their migration to the brain where they initiate a neuropathogenic program; if this activation is aborted by reducing the peripheral virus burden with antiretroviral drugs, then brain disease is reduced. This view suggests that differences in the ability of HIV-1 to activate macrophages or in the host's sensitivity to this activation account for the incomplete penetrance of HAD.

Previous studies by several investigators have shown that HIV-1 gp120 confers a phenotype of macrophage activator upon the virus (Choe *et al*, 2001; Cicala *et al*, 2002; Fantuzzi *et al*, 2001; Giulian *et al*, 1993; Liu *et al*, 2000; Power *et al*, 1998; Wahl *et al*, 1989; Zhang *et al*, 2003). The systems employed were diverse and they reach somewhat different conclusions about viral determinants responsible for the observed phenotype. There are two major classes of effects measured: neuronal cell death and induction of soluble mediators associated with neuropathicity. In a comparison of X4 and R5 virus strains, exposure to HIV-1–induced apoptosis in neurons and macrophages in culture was inversely correlated with the ability to replicate in macrophages (Zheng *et al*, 1999). Expression of inflammatory proteins, tumor necrosis factor (TNF)-α, interleukin (IL)-1β, macrophage imflammatory protein (MIP)-1α and MIP-1β have been shown to be activated by R5 virus or gp120, or in some cases, by both X4 and R5 viruses (Fantuzzi *et al*, 2001; Wahl *et al*, 1989). Comparison of recombinant HIV-1 indicated that virus containing regions of gp120 from demented patients induced macrophages to produce more neurotoxin than did virus containing envelope from nondemented patients (Power *et al*, 1998). Exploring the basis of this neurotoxicity, some such recombinants, including replication-incompetent viruses, killed neurons directly as well as induced macrophages to produce neurotoxic factors (Zhang *et al*, 2003).

Although less is known about strain-specificity of Tat for activation of macrophages, studies have shown that HXB-2 Tat can induce macrophages or microglia to produce neurotoxins, including TNF-α or impair neuronal function through down-regulation of glycogen synthase kinase 3β (Chen *et al*, 1997; D'Aversa *et al*, 2004; Sui *et al*, 2006).

In the present study we employed the assumption that HIV-1 infection and activation of monocytic cells, possibly occurring outside the CNS, are central to neuropathogenesis. We investigated the responses of macrophages to four well-characterized replication-competent R5 HIV-1: ADA (Gendelman *et al*, 1988), B-aL (Gartner *et al*, 1986), DJV (Ghorpade *et al*, 1998), and YU-2 (Li *et al*, 1991). At a fundamental level the viruses are similar in that they employ the same receptors for productive infection of their native target cells. However, we reasoned they might have different potentials for activation of monocyte-derived macrophages (MDMs), because these viruses differ in tissue origin. ADA was isolated from blood cells, B-aL from alveolar lavage, and DJV and YU-2 were isolated from the brains of patients with HAD and encephalitis. Working on the additional assumption that some of the differences in the development of HAD reside in the host, we tested responses by MDMs from several different donors. MDMs were infected in culture with HIV-1 and quantitative real-time polymerase chain reaction (PCR) amplification was employed to detect cellular transcripts and enzyme-linked immunosorbent assay (ELISA) was employed to detect cellular proteins at early and late points after virus exposure. Cellular genes tested included cytokines, chemokines, and interferon-related genes that have been associated with HIV-1 pathogenesis or the responses of macrophages to other viruses. We find that near the peak of productive infection, ADA or B-aL induced the expression of several cellular genes by MDMs in all donors tested. In sharp contrast, MDMs from certain donors were variably susceptible to DJV and YU-2 but responded to these viruses with intense gene expression within the first day of exposure. Thus HIV-1 isolated from highly diseased brain can be distinguished by its activation of MDMs. The heterogeneity of responses among donors indicates that there is a host-specific component to this activation which may contribute to the heterogeneity among individuals in the development of HIV-1–associated brain disease.

Results

MDM responses to different HIV-1 strains

Under the assumption that differences in the activation of macrophages by HIV-1 underlie differences in the development of HIV-1–associated dementia, this study attempts to reproduce some of the natural variability of HIV-1 infection of human beings. Our approach is to compare four distinct R5 HIV-1 and several different donors of MDMs for changes in cellular gene expression, throughout this work cells from different donors are designated alphabetically. We required that all macrophages studied are susceptible to productive infection by HIV-1/ADA to screen out cell populations that impose an overall block on R5 HIV-1 interactions, such as homozygous ΔCCR5 (Fantuzzi *et al*, 2001; Wahl *et al*, 1989). Using cells from donor A, we tested ADA induction of the interferon-related genes, viperin, STAT-1, interferon regulatory factor 7 (IRF-7), as well as the β chemokine MIP-1α and the cytokine IL-1β, which have been associated with HIV-1 neuropathogenesis. Macrophages were infected in culture with ADA and samples were collected 1 day and 7 days later for quantitative PCR (QPCR) and ELISA. QPCR and ELISA are conducted to complement each other and to determine if cellular responses to HIV-1 are visible at both RNA and protein levels. QPCR was conducted in duplicate and throughout this work all duplicates were within 10% of each other, indicating reasonable

reproducibility of assay. QPCR data are expressed as fold-activation versus mock-infected cells from the same donor cultured in parallel—this is the standard format used here to assay RNA expression. Absolute levels of the proteins assayed in ELISA are reported, including the levels in supernatants of mock-infected cells, shown in parentheses in the tables, to permit analysis of the extent of activation. In this experiment infected cells were cultured in the absence and presence of the HIV-1 protease inhibitor saquinavir to block virus spread to distinguish cellular activation arising from HIV-1 infection from any potential contaminating activator, including cellular products of infected macrophages that might cosediment with virus particles (Figure 1). HIV-1 p24 production was fully sensitive to exposure to saquinavir. Consistent with previous studies, MIP-1α was induced upon virus exposure and also at the peak of infection (Fantuzzi *et al*, 2001; Wahl *et al*, 1989), all other genes were induced only at the peak of infection. The activation due to HIV-1 exposure was sensitive to inhibition by saquinavir, forging the link between cellular activation and ADA replication.

We then exposed macrophages from three different donors (donors B, C, D) to ADA, B-aL, and DJV and sampled cells for the extent of cellular gene activation (Table 1). Near the peak of productive infection by ADA or B-aL, day 7 after virus exposure, expression of individual transcripts or groups of cytokines, adhesion factors, or interferon-related genes were

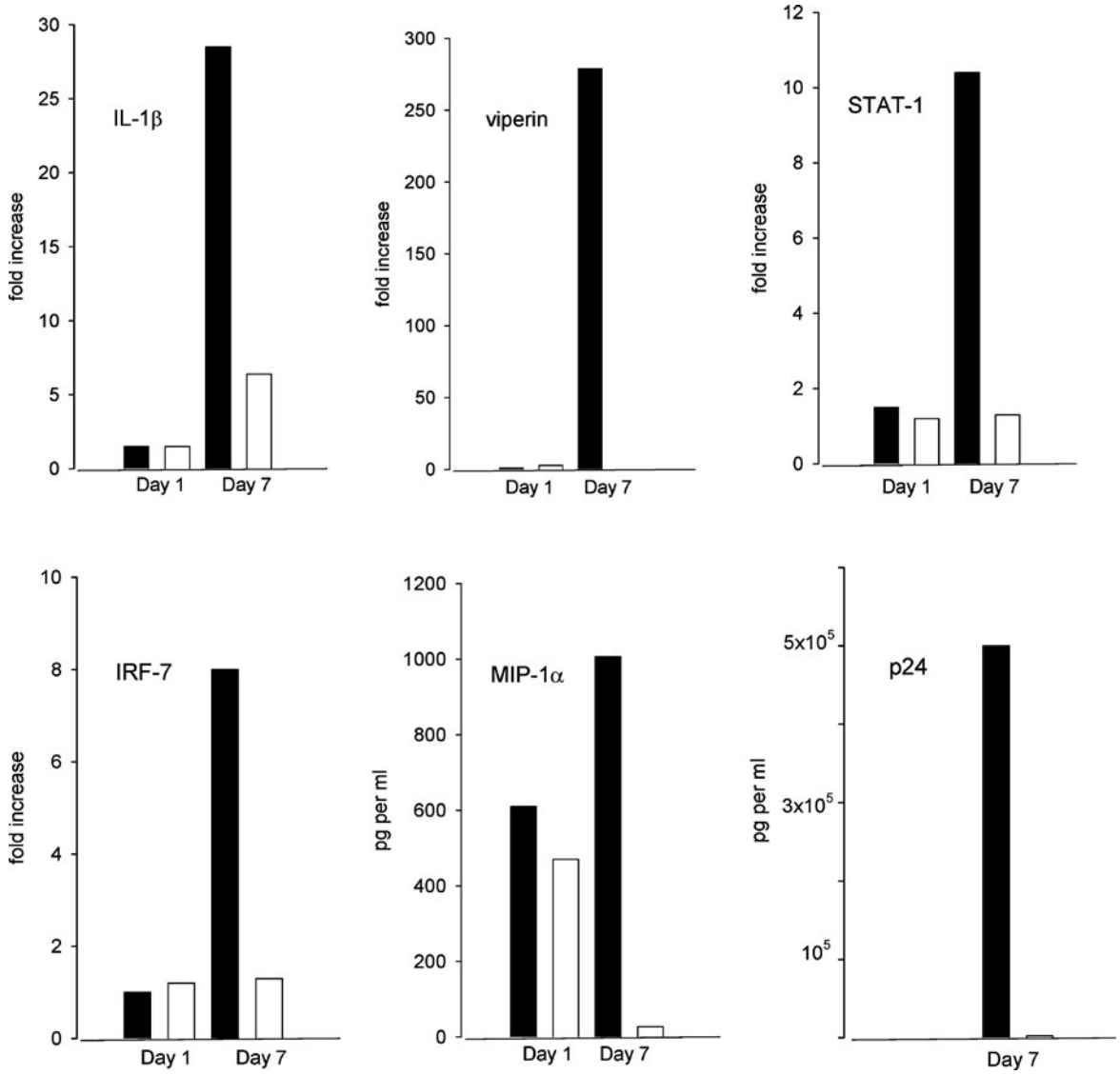

Figure 1 MDM response to ADA and its inhibition by the protease inhibitor, saquinavir. MDM RNA and cell supernatants were harvested one and seven days after ADA infection in the absence (*closed bars*) and the presence (*open bars*) of saquinavir. The extent of induction of transcripts encoding IL-1β, viperin, STAT-1, and IRF-7 is expressed as fold induction relative to transcripts in mock-infected cells cultured and treated in parallel. ELISA was performed to assay extracellular MIP-1α and p24 and is presented as pg per ml cell supernatant.

Table 1 Induction of gene expression by ADA, B-aL, or DJV infection of MDMs[a]

	ADA		*B-aL*		*DJV*	
Gene product[b]	*D1*	*D7*	*D1*	*D7*	*D1*	*D7*
			Donor B			
IL-1β	4.6	3.9	1.2	5.3	317.5	3.9
IL-6	ND[c]	2.0	3.0	8.0	363.7	99
IL-8	ND	15.0	−1.6	10.0	6.1	60
CCL-1	1.6	1.0	−2.8	1.3	13.7	−3.0
COX-2	ND	3.3	−1.6	2.5	7.7	2.7
MMP-1	ND	9.0	1.1	3.6	17.4	1.0
Siglec-1	ND	2.5	−2.0	3.5	12.8	9.8
Viperin	9.4	75.6	1.6	45.4	918.7	45.4
IRF-7	3.0	4.5	−1.3	2.8	27.5	9.1
IFIT-1	9.5	15.4	1.0	5.5	127.3	15.6
IFI-78K	17.7	18.6	1.7	6.0	211.9	37.7
OAS-1β	ND	5.1	−1.2	4.3	17.2	10.7
STAT-1	2.1	4.6	−1.2	2.4	6.2	9.9
MIP-1α	880 (130)	6800 (290)	280	5800	27,000	710
TNF-α	100 (<25)	<25 (<25)	50	<25	>800	>800
p24	ND	630,000	ND	730,000	ND	2000
			Donor C			
IL-1β	1.1	−2.5	−1.6	−2.5	32.5	−2.5
IL-6	ND	−5.0	1.4	1.3	192.6	2.1
IL-8	ND	−2.0	−1.3	2.0	12.6	2.5
CCL-1	1.1	24	−2.6	18.0	32.5	12
COX-2	ND	1.0	−1.5	5.2	11.9	4.4
MMP-1	ND	−4.8	1.3	2.3	8.0	10.0
Viperin	−1.1	154.4	−1.5	186.0	299.9	5.5
IRF-7	−1.5	9.0	−1.5	15.6	22.0	3.4
IFIT-1	1.0	87.8	−1.9	83.8	180.2	4.9
IFI-78K	−1.1	40.7	−2.1	39.5	44.8	5.6
OAS-1β	ND	4.7	−1.1	10.3	28.8	3.3
STAT-1	−1.1	8.0	−1.8	9.6	8.5	3.3
MIP-1α	24,000 (20,000)	5300 (470)	19,000	16,000	110,000	570
TNF-α	980 (850)	51 (<25)	550	110	42,000	150
p24	ND	2,600,000	ND	1,800,000	ND	250
			Donor D			
IL-1β	−1.1	5.0	−1.2	2.5	3.6	1.0
IL-6	2.3	12.5	1.3	−1.3	88.5	7.5
IL-8	1.2	3.3	−1.5	3.3	1.7	10.0
CCL-1	−1.2	3.0	−2.0	4.0	7.7	−4.0
COX-2	1.8	2.0	−1.1	1.5	12.8	1.0
MMP-1	−1.5	3.5	1.1	2.5	1.4	−2.0
Siglec-1	1.2	3.9	−2.0	3.8	5.9	8.6
Viperin	9.3	39.7	1.3	17.3	939.4	7.6
IRF-7	1.2	6.3	−3.3	3.6	8.1	6.4
IFIT-1	8.3	17.2	1.1	7.1	451.5	3.7
IFI-78K	6.5	11.1	−1.2	6.9	164.3	10.8
OAS-1β	1.4	3.6	−2.5	3.5	23.2	4.6
STAT-1	1.1	2.7	−2.5	2.2	1.6	3.0
MIP-1α	ND	640 (26)	ND	380	ND	490
TNF-α	500 (240)	78 (100)	410	96	21,000	130
p24	ND	630,000	ND	1,300,000	ND	730

[a]Cells from donors B, C, and D were infected as indicated.
[b]The data are the average fold change of the indicated transcripts relative to the levels from mock-infected MDMs from the same donor at the same time point after infection, except for MIP-1α, TNF-α and p24; the extracellular levels of these three proteins in pg/ml are given, values from mock-infected cells are shown in parentheses.
[c]ND = not determined.

increased, with two exceptions. Cells from all donors shown in this work expressed TNF-α at consistently higher levels on day 1 than on day 7 after virus exposure and donor C's cells also expressed higher levels of MIP-1α at this early point. Cells from all three donors had a markedly different response to DJV. Unlike ADA and B-aL, DJV was unable to productively infect these MDMs. Nevertheless exposure to DJV induced a very high level of expression of most of the genes tested and this induction was observed on day 1 after virus exposure, prior to virus expression. The difference in response to ADA and B-aL on the one

Table 2 Alternative response to DJV infection by MDMs[a]

	Donor E				*Donor F*			
	ADA		*DJV*		*ADA*		*DJV*	
Gene product	*D1*	*D7*	*D1*	*D7*	*D1*	*D7*	*D1*	*D7*
IL-1β	4.2	1.0	1.0	1.6	1.3	9.0	1.1	4.4
IL-8	ND	ND	ND	ND	1.5	74.1	2.0	12.9
CCL-1	4.7	15.0	1.0	1.5	1.2	25.5	1.6	3.3
COX-2	ND	ND	ND	ND	1.2	7.1	2.8	8.2
Viperin	2.5	61.3	−1.2	2.5	1.8	253.6	2.8	30.1
IRF-7	1.3	14.8	1.5	4.2	1.3	14.2	2.4	4.3
IFIT-1	1.0	18.4	−1.1	2.1	1.1	22.3	2.4	7.8
STAT-1	1.1	4.4	1.0	1.4	1.1	9.4	1.3	2.6
MIP-1α	22,000 (3400)	5300	3500	1100	ND	ND	ND	ND
TNF-α	2,900 (710)	650 (81)	800	120	<25	<25	<25	<25
p24	ND	1,800,000	ND	460,000	ND	2,800,000	ND	2,000,000

[a]See Table 1 footnotes.

hand and DJV on the other hand was highly significant ($P < .001$) and could be observed among cytokines, chemokines, and interferon-related genes.

MDMs from another two donors (donors E and F) were infected by ADA or DJV and both viruses infected efficiently, permitting investigation of the response to DJV by cells that were highly susceptible to the virus (Table 2). Once again, ADA serves as a positive control for productive infection and cellular activation. The response to ADA by cells from donors E and F was basically like that of the other donors: moderately high induction correlated to p24 production. What is striking is the comparison of responses to DJV by cells from donors E and F (Table 2) to that of donors B, C, and D (Table 1). The extent of induction of each cellular gene is significantly lower in the former pair that permitted virus replication than the later trio, in which DJV was poorly productive ($P < .001$) and the induction observed was greater on day 7 than day 1 after infection, the pattern observed with ADA and B-aL. Actually, cells from donors E and F are significantly more responsive to ADA than to DJV ($P < .001$). These findings indicate that despite their common susceptibility to HIV-1 activation and infection, hosts differentially respond to neuropathogenic HIV-1.

DJV was isolated from the brain of a patient with HAD. To investigate whether its cellular gene induction is characteristic of other HIV-1 from the brain, we investigated the macrophage response to YU-2 in comparison to our reference, ADA (Table 3). Like DJV, YU-2 induced very large responses early after

Table 3 Induction of gene expression by ADA or YU-2 infection of MDMs[a]

	Donor G			
	ADA		*YU-2*	
Gene product[b]	*D1*	*D7*	*D1*	*D7*
IL-1β	1.1	−1.5	28.2	−10.0
IL-6	7.7	UN	6.1	UN
IL-8	−1.2	−1.5	6.8	1.7
CCL-1	1.4	4.0	34.0	−1.5
COX-2	−1.5	1.2	24.6	2.4
MMP-1	1.1	1.0	71.3	2.5
Siglec-1	−1.3	70.4	20.6	1.3
Viperin	−2.0	28.9	1633	4.0
IRF-7	−3.3	6.6	30.3	1.6
IFIT-1	−2.5	38	243.8	2.2
IFI-78K	−1.3	50.0	475.7	2.6
OAS-1β	−5.0	10.1	38.6	2.0
STAT-1	−2.0	4.8	7.6	1.6
IL-6[c]	25 (<5)	75	695 (<5)	611 (53)
MIP-1α	11,000 (2400)	710 (450)	22,000 (2900)	1,300 (470)
TNF-α	8000 (910)	ND	37,000 (870)	ND
p24	ND	510,000	ND	40,000

[a]Cells from donor G were infected as indicated.
[b]See Table 1 footnotes.
[c]The extracellular levels in pg/ml are given, values from mock-infected cells are shown in parentheses.

infection, whereas the responses to ADA were higher during productive infection as shown in Tables 1 and 2. Similar rapid responses to YU-2 were observed with cells from three other donors. Taken together, these findings illustrate that the same cell population responds differently to different R5 HIV-1 and that a single virus strain can activate HIV-1 susceptible MDMs from different hosts differently.

Specificity of the activation of MDMs by HIV-1
All of the viruses used here employ CD4 and CCR5 for productive infection of MDMs, but we observe that DJV activates cells from some donors and not from others. The simplest explanation for this observation is that early activation characteristic of DJV and YU-2 is transduced by an alternative receptor displayed in a host-specific manner and that ADA and B-aL fail to recognize this receptor. To explore the explanation that the activating receptor used for signaling by YU-2 is ignored by B-aL, we tested whether a 10-fold excess of B-aL competes with YU-2 and blocks its early activation of MDMs (Figure 2). Extracellular TNF-α was measured 6 h after infection and extracellular p24 was measured 7 days after infection. YU-2 was able to induce cytokine synthesis despite the presence of competing B-aL; in contrast, B-aL induced little TNF-α; both viruses productively infected the cells. Similar findings were obtained in MDMs from two other donors. These findings are consistent with the possibility that receptors different from CD4 and CCR5 mediate the immediate response of MDMs to YU-2 and DJV. In a preliminary study, we tested whether anti-CCR5 monoclonal antibody that neutralizes productive infection by R5 viruses had any effect upon the early induction of TNF-α expression by YU-2. MDMs were mock infected, infected by YU-2, infected in the presence of anti-CCR5, or infected in the presence of control murine immunoglobulin G (IgG) secreting 306, 44,600, 52,500, and 42,800 pg TNF-αper ml, respectively. Although these findings support the possibility of an activating receptor for YU-2 different from CCR5, more studies are clearly required.

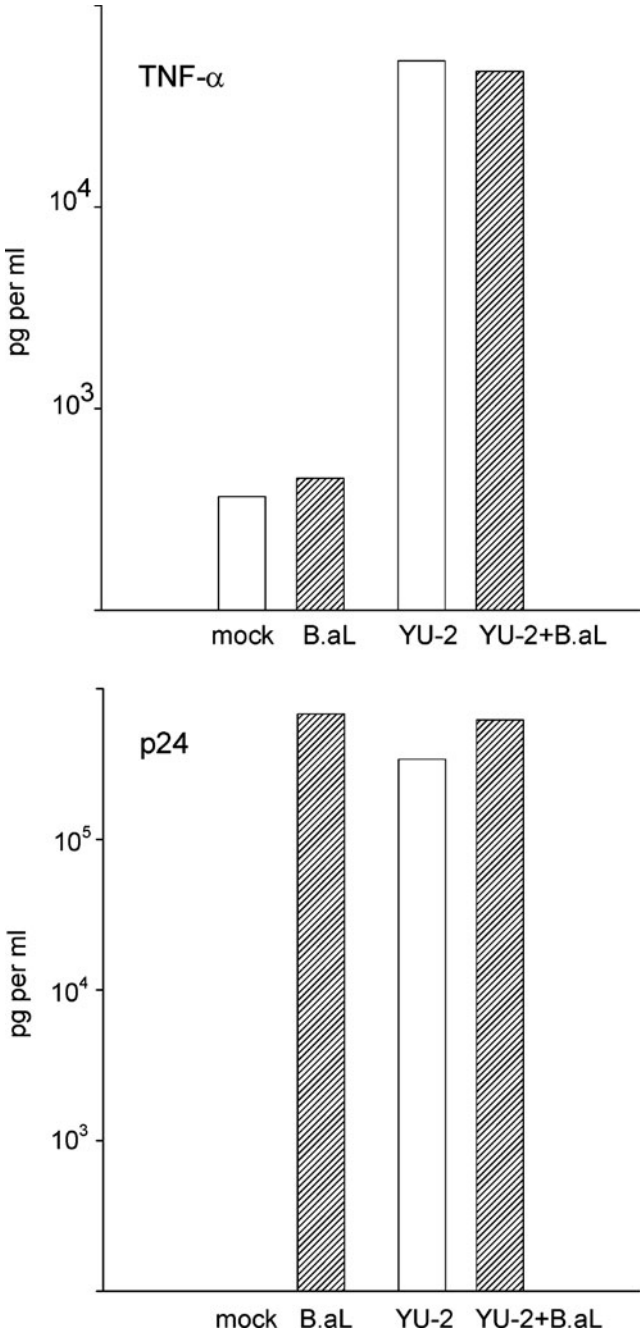

Figure 2 MDM response to YU-2 and B-aL. MDMs were infected with YU-2 at 0.02 pg p24 per cell or with B-aL at 0.2 pg p24 per cell or with both viruses at these doses. Cell supernatants were harvested 6 h later for assay of TNF-α by ELISA (*upper panel*) or 7 days later for assay of viral p24 by ELISA (*lower panel*).

Discussion

The results reported here indicate that HIV-1 strains isolated from an inflammatory environment in the brain can be distinguished from some other R5 HIV-1 by their ability to activate early gene expression of MDMs from select donors. These observations join those of other investigators who have linked potentially neuropathogenic HIV-1 to their tropism or to the responses they induce in culture (Gorry *et al*, 2001; Peters *et al*, 2004; Power *et al*, 1998; Smit *et al*, 2001). We also report differences in responses among donors that may reflect part of the observed variation in the development of brain disease among HIV-1–infected persons.

Here, ADA infection of MDMs constitutes a common and reproducible system to investigate innate immune responses of cells from several donors to productive HIV-1 infection, as previously described (Bukrinsky *et al*, 1995). Observation of this generalized response to an R5 virus ensures that cells from each host studied displayed the essential receptors for virus entry and carried no mutations that might impair HIV-1 replication or response (O'Brien and Moore, 2000). The inhibition of responses to ADA by

treatment of infected cells with the HIV-1 protease inhibitor confirms that the cell gene induction observed arises specifically from HIV-1 replication. The gene products monitored were chosen to reflect different aspects of the virus-cell interaction. We detected induction of cytokines and chemokines involved in viral pathogenesis or progress of virus infection. For example, TNF-α and IL-1β have frequently been associated with responses of macrophages to HIV-1 in culture (Vázquez *et al*, 2005) and in neuropathogenesis in infected persons (McArthur *et al*, 2003). CCL1 has been shown to be induced by exposure to IL-1β and to serve as a competitive inhibitor of HIV-1 infection (Horuk *et al*, 1998; Selvan *et al*, 1997). IL-6 is expressed in the brain upon simian immunodeficiency virus (SIV) infection and has been implicated in animals as a predictor of development of SIV encephalitis (Mankowski *et al*, 2004; Roberts *et al*, 2004). MIP-1α is a competitive inhibitor of R5 HIV-1 infection and was shown to be induced in culture by HIV-1 exposure in several previous studies (Choe *et al*, 2001; Fantuzzi *et al*, 2001).

The second large group assayed are interferon-related genes, which both mediate antiviral activity throughout the HIV-1 life cycle and serve as sensitive indicators of viral infection (Meylan *et al*, 1993). Viperin has been shown to be a potent human antiviral factor, antagonizing cytomegalovirus infection (Chin and Cresswell, 2001). STAT-1 has been identified in objective searches for gene induction by SIV in the brain (Roberts *et al*, 2003) and is induced *in vitro* and *in vivo* in macrophages in the CNS in an feline immunodeficiency virus (FIV) model of neurotoxicity (Zhu *et al*, 2005). STAT-1, viperin, OAS-1, IRF-7, and IFIT-1 was also found to be induced in human cells by JC virus infection in culture (Verma *et al*, 2006). IRF-7 is the pivotal factor in regulating the large family of interferon-related genes (Honda *et al*, 2005). Moreover, STAT-1 and IL-6 (interferon-β) have been shown to act collaboratively and exert positive feedback upon their own expression as well as the expression of their target genes (Kim *et al*, 2002).

Many of the genes we selected to evaluate have been shown to be induced in culture by HIV-1 gp120 or Tat (Chen *et al*, 1997; Choe *et al*, 2001; D'Aversa *et al*, 2004; Fantuzzi *et al*, 2001; Kohler *et al*, 2003). Our experimental scheme was designed to capture responses to all viral products, because we sample cells near the time of virus binding through gp120 as well as near the peak of infection. In the case of ADA and B-aL, expression of the cellular genes tested coincided with the peak of virus expression, with the exception of TNF-α. Although use of the specific HIV-1 protease inhibitor, saquinavir, clearly identifies HIV-1 replication and not any other agent as the stimulus we assess, we have not identified the viral product(s) responsible for the gene induction observed. Tat, gp120, Nef, Vpr (Chen *et al*, 1997; Choe *et al*, 2001; D'Aversa *et al*, 2004; Fantuzzi *et al*, 2001; Kohler *et al*, 2003; Swingler *et al*, 1999; Vázquez *et al*, 2005), and other viral products can each contribute to the activation by most R5 HIV-1 observed near the peak of infection. There seems to be some strain specific component to this activation as well, because ADA and B-aL tend to induce higher responses than did DJV and YU-2 during productive infection; however, further studies are required to clarify this point.

A reasonable candidate for the virus strain–specific early activating determinant of YU-2 and DJV is gp120, because envelope binding precedes new synthesis of viral products. We made an initial attempt to determine whether YU-2 employs the same receptors as does B-aL in its cellular induction by exposing cells to YU-2 in the presence of excess B-aL. If YU-2 employs the same receptor for induction as does B-aL, then excess B-aL should attenuate the YU-2 activity. However, we detected no effect of B-aL upon YU-2 induction of TNF-α, raising the possibility that YU-2 employs an alternative receptor for macrophage activation at early timepoints. An alternative possibility is that YU-2 and DJV have a higher avidity for CD4-CCR5 complexes than do ADA and B-aL. However, if this were the case, then all host macrophages that are susceptible to R5 HIV-1 should behave similarly, and we observe that the early pathway of cellular activation is host dependent. This is shown most clearly in the failure of cells from donors E and F to respond to DJV by early activation despite their highly productive infection by the virus. The most provocative conclusion of our studies is that there is some heterogeneity among human beings for display of the hypothetical activating receptor recognized by DJV and YU-2.

Based upon our results and those of others, we can sketch a view of HIV-1 activation of macrophages. During infection of macrophage from many individuals, R5 HIV-1 signals through gp120 during binding, albeit not extensively. Once infection is established, several of the newly synthesized viral proteins each activate cellular gene expression. However, macrophages from other individuals display a receptor for a virion component found only in a subset of HIV-1 strains. Early in infection, this virion-receptor binding sets up a signaling cascade, yielding high level cellular gene expression. HIV-1–infected individuals whose macrophages carry this hypothetical activating receptor are at an enhanced risk of neuropathogenesis.

Conclusions

During productive infection of primary macrophages, R5 HIV-1 activates the expression of several cellular genes associated with antiviral defense. However, only HIV-1 strains associated with dementia and encephalitis activate intense and early gene expression and then only in macrophages from a subset of donors. This restricted response may reflect part of the observed variation in the development of brain disease in AIDS.

Methods

Cells, viruses, and infection
Human monocytes were prepared from peripheral blood mononuclear cells of HIV-1 and hepatitis B virus–negative donors by countercurrent centrifugal elutriation. Monocytes were induced to adhere and differentiate to macrophages (MDMs) by culture at 5.0 $\times 10^6$ cells per T25 flask, 2.5 $\times 10^6$ cells per well in 6-well plates, or 6.0 $\times 10^5$ cells per well in 24-well plate in Dulbecco's modified Eagle's medium (DMEM) with 10% endotoxin-free, heat-inactivated human serum (Cambrex, East Rutherford, NJ), 10% giant cell tumor conditioned medium (BioVeris, Gaithersburg, MD), 2 mM glutamine, and antibiotics. Cells were cultured for 5 to 7 days until fully adherent, thereafter they were cultured in DMEM with 10% endotoxin free fetal bovine serum, glutamine, and antibiotics. HIV-1 ADA, DJV, and B-aL were prepared by cell-free virus infection of macrophages and collection of extracellular virus 7 to 14 days after infection. HIV-1 YU-2 was prepared by proviral DNA transfection of 293T cells and collection of extracellular virus. Culture supernatants of mock-infected macrophages (for ADA, DJV, and B-aL) or 293T cells (for YU-2) were used as controls for the inoculations. Culture supernatants of infected cells and controls were concentrated by centrifugation at 12,000 $\times g$ for 2 h at 4°C, resuspended in phosphate-buffered saline (PBS), and frozen at −80°C until use. Viral stocks were quantified for p24 antigen level using Coulter HIV Ag kit (Hialeah, FL) according to manufacturer's instructions. Viral stocks were found to be free of mycoplasma by MycoAlert detection kit (assay) (Cambrex). For activation by HIV-1, fully differentiated MDMs were exposed to ADA, DJV, and B-aL at 0.1 pg p24 per cell, to YU-2 at 0.02 pg p24 per cell, for 1 h at 37°C, medium was changed, and cells were incubated a further 5 or 23 h prior to harvest. Concentrated cell supernatants for mock infection were used at the same dilution as virus stocks. Alternatively, for assay of long-term responses to HIV-1, MDMs were exposed to HIV-1 for 1 h, medium was changed, and cells were cultured for 1 week, with medium replacement as needed prior to sample collection. In the indicated experiments, MDMs were pretreated with the HIV-1 protease inhibitor saquinavir (Roberts *et al*, 1990) (5 μM) or mouse IgG (Sigma, St. Louis, MO) or monoclonal anti-human CCR5 antibody (20 μg/ml) (R&D Systems, Minneapolis, MN), for 1 h prior to and during stimulation. Cells were cultured for 1 week in the presence of saquinavir and samples were harvested as described.

ELISA
Supernatants harvested during macrophage activation were subjected to ELISA for TNF-α (Cell Sciences, Norwood, MA), MIP-1α (R&D Systems), and quantified for p24 antigen level using Coulter HIV Ag kit (Hialeah, FL) according to manufacturers' instructions. The limits of detection of the ELISA for p24 is 5 pg and for TNF-α and MIP-1α 25 pg per ml; data reported as <or> a value in the tables were outside the standard curve of the assays.

RNA preparation and gene expression analysis by quantitative real-time PCR (QPCR)
MDMs from seven different donors were used for QPCR. Total RNA was prepared from cultures of MDM either 6 h, 24 h, or 7 days after virus exposure using the RNAeasy total RNA extraction kit (Qiagen, Valencia, CA). RNA quality was assessed using spectrophotometric analysis and gel electrophoresis prior to cDNA synthesis. One hundred nanograms of total RNA from each sample was used to generate a high fidelity cDNA (NuGen Technologies, San Carlos, CA) according to manufacturer's instructions. For donor E, cDNA was generated from 1 μg of total RNA following the protocol for Superscript (Invitrogen, Carlsbad, CA). QPCR was conducted using Taqman chemistry with probes and primers designed using Primer Express v.1.0 (ABI, Foster City, CA). Following probe and primer optimization all cDNAs were diluted and used in a 25-μl PCR reaction containing 12.5 μl of ABI 2× Universal Master Mix (Roche, Nutley, NJ), 2.25 μl of each forward and reverse primers (final concentrations 900 nM), 1.25 μl of probe (final concentrations 100 nM), and RNase/DNase-free water. All reactions were performed in duplicates and were run in an ABI 7500 with the following cycle parameters: 1 cycle of 50°C (2 min) followed by 95°C (10 min), 40 cycles of 95°C (15 s) followed by 60°C (1 min). Raw data were analyzed using the Sequence Detection Software (ABI), and relative quantitation using the comparative threshold cycle method was performed in RQ study Software (ABI). The differences in gene induction by a given virus in a given host cell population compared to gene induction by a different virus in the same host cell population were evaluated for statistical significance using a χ^2 test and are reported in the text.

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