Cellular Gene Expression Profiles in Rhesus Macaques Challenged Mucosally with a Pathogenic R5 Tropic Simian Human Immunodeficiency Virus Isolate

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Abstract

Insights into the host factors that contribute to an effective antiviral immune response may be obtained by examining global gene expression in simian human immunodeficiency virus (SHIV)-infected nonhuman primates that exhibit different virological outcomes. Immune responses and gene expression profiles in peripheral blood mononuclear cells (PBMCs) were compared between animals that controlled or did not control viremia after infection. Rectal inoculation of eight rhesus macaques with R5-tropic SHIV_{SF162P3} resulted in a high level of plasma viremia during the acute phase of infection. The viremia was controlled to below levels of detection in six of these animals at the set point (controllers), whereas two animals had persistent viremia throughout the 140 wk that the animals were monitored (non-controllers). CD4⁺ T-cell counts declined slightly in both controllers and non-controllers in the acute phase of infection, but CD4⁺ T-cell counts continued to decline only in the non-controllers. Neutralizing antibodies to the challenge virus were variable and could not account for the control of viremia. However, analysis of the cellular gene expression profiles in the PBMCs from both groups of animals revealed distinctive gene expression patterns between controllers and non-controllers. Using the paired LPE test, 59 genes with *p* values <0.01 were identified and specific differences in the gene expression profiles in PBMCs from controllers versus non-controllers were detected.

Introduction

ALTHOUGH HIV-1 INFECTION HAS BEEN SHOWN to cause a gradual decline of CD4⁺ T cells and progressively impair host immune functions, a small number of HIV-1-infected individuals, called long-term non-progressors (LT-NPs), naturally suppress viral replication and maintain normal CD4⁺ T-cell numbers and immune functions for over 10 y in the absence of antiretroviral therapy (27). It has been suggested that immune mechanisms such as neutralizing antibodies and HIV-specific T-cell immune responses (including virus-specific cytotoxic T lymphocytes [CTLs], CD8-derived non-lytic suppressor factors, and CD4⁺ T-cell proliferative responses) could play a role in the control of HIV-1 infection in LT-NPs (24,33,34).

Chimeric simian human immunodeficiency virus (SHIV), consisting of a SIV backbone and selected HIV-1 genes, has

significantly extended the use of macaques to examine HIV-1 vaccines (13). Typically, macaques elicit neutralizing antibodies and CTL responses to SHIV and have been used to study the effects of inducible cytokines and chemokines on virus infection and replication (9). It also has been demonstrated that infection of rhesus macaques with the R5-tropic SHIV_{SF162P3} results in varying levels of viremia, especially in the chronic phase of infection, which is similar to that observed in HIV-1-infected humans (10,14,15).

Microarray technology has facilitated a more thorough and comprehensive analysis of the modulation of cellular gene expression profiles in HIV-1/SIV infection. Recently, global gene expression profiles revealed that a number of genes involved in transcription regulation, RNA processing and modification, and protein trafficking were upregulated in resting CD4⁺ T cells of viremic patients compared to those of aviremic patients (5). Other studies have examined gene

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expression patterns in HIV-1-infected cell lines to understand biochemical changes related to virus replication and cellular restriction (18,32). In addition, gene expression profiling of immunologically relevant genes was also conducted using the SIV-infected nonhuman primate model to characterize both acute and progressive phases of infection. It was observed that several interferon-stimulated genes were up-regulated in response to infection (4,31). The identification and characterization of host factors that modulate the virological status in HIV-1-infected humans in both acute and chronic phases of infection not only broadens our understanding of virus-host interactions, but also may lead to the identification of new and effective therapeutic and preventive approaches.

Since the virological profile in SHIV_{SF162P3}-infected macaques resembles that of HIV-1-infected humans, the evaluation of global cellular gene expression profiles in SHIV-infected macaques could shed light on the molecular mechanisms involved in the control of viremia. To test this hypothesis, global gene expression profiles in PBMCs were examined in rhesus macaques that either did or did not spontaneously control infection with SHIV_{SF162P3}. Although the numbers of animals used in this study are small, differences in gene expression profiles in PBMCs between the two groups of animals are consistent with results from related studies (5,7,31), and have identified genes of unknown function that merit further investigation.

Materials and Methods

Preparation of challenge virus and rectal challenge of rhesus macaques

SHIV_{SF162P3} (Aaron Diamond, New York, NY) was inoculated intravenously into a rhesus macaque. PBMCs were isolated from the virus-inoculated animal on day 14 post-infection, when plasma viremia peaked to a level of 10^8 copies/mL, enriched for CD4⁺ T cells by negative selection with magnetic beads (Dynal Inc., Camarillo, CA), coated with anti-CD8 antibodies according to the manufacturer's protocol, and activated with phytohemagglutinin (PHA) for 48 h. These activated CD4⁺ T-cell-enriched PBMCs were then co-cultured with PHA-activated naïve rhesus PBMCs to isolate virus. Cell-free supernatant was monitored for SIV p27 and harvested when the p27 content was >72 ng/mL. The supernatant was filtered and cryopreserved as 1-mL aliquots in the vapor phase of liquid nitrogen. The virus stock was shown to have an *in vitro* infectious titer of approximately 5000 TCID₅₀/mL in rhesus PBMCs.

Eight rhesus macaques (*Macaca mulatta*) of Indian origin weighing 4–5 kg each that were virologically and immunologically negative for type D retrovirus, SIV, and simian T-lymphotropic virus were used for the challenge with SHIV_{SF162P3}. Animals that were used for global gene expression profiles were Mamu A*01 negative. Rhesus macaques were inoculated rectally with the SHIV_{SF162P3} stock in 1 mL of RPMI medium containing 5000 (713L and 714L), 2500 (717L and 718L), 500 (719L and 720L), or 100 (68M and 69M) TCID₅₀. The Institutional Animal Care and Use Committee of Advanced BioScience Laboratories approved all protocols used in this study.

Virological assays

The animals were bled periodically following challenge, and the viral RNA load in plasma was assessed using a sensitive nucleic acid sequence-based amplification assay (NASBA) to quantitate SIV RNA (25). The assay used for the study had a lower limit sensitivity of 500 viral RNA copies per milliliter of plasma. To confirm virus transmission, PBMCs collected from animals 21 d post-infection were subjected to qualitative virus isolation by co-culturing with PHA-activated human PBMCs (21).

Neutralizing antibody

Neutralization assays using sera from SHIV_{SF162P3}-infected animals against the challenge virus were performed in U373 cells expressing a β -galactosidase reporter gene under HIV-1 LTR promoter control as described elsewhere (22). Briefly, serial dilutions of heat-inactivated infected serum were incubated with 200 TCID₅₀ of SHIV_{SF162P3} for 60 min at 37°C and the mixture was then added to U373 cells coated onto 96-well plates. The culture was incubated for 48 h. The cells were then thoroughly washed and fresh medium was added to each well. Viral infection was quantified based on expression of the β -galactosidase using the Galacto-Start One-Step Galactosidase Reporter Gene Assay System (Applied Biosystems, Foster City, CA) according to the manufacturer's protocol.

Flow cytometry for CD3⁺/CD4⁺ T-cell counts

CD4⁺ T-cell numbers were determined using a FACScan flow cytometer (BD Biosciences, San Jose, CA). Briefly, the cells were stained with monoclonal antibodies, CD3 FITC (Pharmingen, San Diego, CA), and CD4 PE (Caltag Laboratories, South San Francisco, CA), and analyzed by flow cytometry using CellQuest software (BD Biosciences, San Jose, CA).

*Mamu A*01 typing*

Mamu-A*01 haplotyping was performed by sequence-specific PCR amplification of genomic DNA using Mamu-A*01-specific primers as previously described (17,20). Briefly, genomic DNA was isolated from rhesus macaque blood using the Promega Wizard Genomic DNA Purification Kit (Promega, Madison, WI). Quantitation of DNA was performed by OD260 absorbance measurements. PCR amplification of genomic DNA (100 ng) was performed using the PCR Master Mix kit (Roche Diagnostics, Pleasanton, CA) and the following conditions: 95°C for 3 min (1 cycle); 95°C for 30 sec, 66°C for 40 sec, 72°C for 40 sec (35 cycles); and 72°C for 3 min (1 cycle). PCR products (685 bp) were resolved by agarose (1%) gel electrophoresis followed by ethidium bromide staining and imaging using the UVP BioImaging System Epichem3 Darkroom (UVP, LLC, Upland, CA).

Animal selection and sample collection for microarray

The selection criteria of animals from the eight infected animals for use in the gene expression profiling were based on the plasma viral RNA loads and CD4⁺ T-cell counts in the acute phase and in the chronic phase of infection. For the

gene expression profiles, four of the eight infected animals were assigned to two groups based on the availability of the animal: controllers ($n = 2$) were animals that had high plasma viral RNA loads in the acute phase of infection, but below 500 copies viral RNA/mL of plasma in the chronic phase of infection; and non-controllers ($n = 2$) were animals that had high plasma viral RNA loads both in the acute and in the chronic phase of infection. Peripheral blood samples were collected in the acute phase (21 d post-infection), and in the chronic phase (approximately 2 y post-infection) of infection, and PBMCs were separated by Histopaque-Ficoll (Sigma, St. Louis, MO) gradient centrifugation. The cells were cryopreserved and stored in the vapor phase of liquid nitrogen until use.

Preparation of cRNA, array processing, and analysis

Total RNA was isolated from PBMCs using Trizol (Invitrogen, Carlsbad, CA) and RNeasy (Qiagen, Valencia, CA) procedures as outlined by the manufacturer. Experimental procedures for GeneChip were performed according to the Affymetrix GeneChip Expression Analysis Technical Manual (Affymetrix, Santa Clara, CA) and as described previously (23). Briefly, double-stranded cDNA was synthesized from total RNA with SuperScript (Life Technologies, Inc., Carlsbad, CA) and a T7-(dT) 24 (GENSET) primer. *In vitro* transcription was performed on the cDNA to produce biotin-labeled cRNA with an Enzo Transcription Kit (Enzo, Farmingdale, NY) as described by the manufacturer. Biotin-labeled cRNA was cleaned with an RNeasy Mini Kit (Qiagen), fragmented to 50–200 nucleotides, and then hybridized to Affymetrix HG-U133A and HG-U133A2.0 GeneChips. These chips were used throughout because rhesus macaque-specific GeneChips were not available when this study was initiated. It has been suggested that the use of human gene chips to evaluate macaque sequences is appropriate because there is a similar linear relationship between the average difference fluorescence for all genes scored in the macaque cRNA sample (23% of all genes on the chip), and the human cRNA sample (28% of all genes) (31). The arrays were then processed on the Affymetrix fluidics station and scanned on an HP GeneArray scanner (Affymetrix, Santa Clara, CA). The intensity for each probe set of the array was captured and preprocessed with RMA (robust multichip averaging) software for the Affymetrix HG-U133A (controller 718 and non-controller 720), and HG-U133A2.0 (controller 714 and non-controller 713) GeneChips (3).

The statistical discovery of differentially expressed genes between two controller and two non-controller animals was performed with the paired local pooled error (LPE) test. The paired LPE test, a recent statistical method that is specialized for the analysis of small-sample microarray data (16), was used because the microarray data were highly correlated within the same platform of Affymetrix GeneChips, but not between the two slightly different Affymetrix platforms, mainly due to their separate preprocessing procedures in the data acquisition. Each animal's samples from two time points (21 d and 2 y) were analyzed either separately or combined as biological replicates, especially the latter to discover the genes consistently up- or downregulated between the two groups. The identified genes with their significant differential expression were further investigated for their overall ex-

pression patterns and associations by hierarchical clustering analysis, and by a pathway database such as Ingenuity Pathway Analysis (Redwood City, CA).

Real-time reverse transcriptase polymerase chain reaction (RT-PCR)

Total cellular RNA was isolated with an RNeasy kit as described by the manufacturer (Qiagen). Primers were designed based on human gene sequences with Primer Express Software (Applied Biosystems, Foster City, CA) and amplified with a 100-bp to 200-bp amplicon for rhesus macaque ADAR (rADAR) rIFI44, and rSTAT1 genes. The human ADAR (hADAR) primers used were, forward: 5'-ATTGTGCC-TACGTGGGAT-3' and reverse: 5'-ATGGGCTGCAGGAAG-TG-3'; hIFI44: forward; 5'-CTGCCTTGAGAACTTATGA-3', reverse: 5'-GCCCTTGGAAAACAGACC-3'; hSTAT1, forward: 5'-CTGCCTTGAGAACTTATGA-3', reverse: 5'-GCC-CTTGGAAA ACAGACC-3'. hGAPDH primers were used for endogenous control. The RT reaction was carried out in duplicate using 1 μ g of total RNA from the naïve animals described above. PCR reactions were performed using 2X SYBR Green PCR Mastermix (Applied Biosystems). The 25 μ L of PCR mixture contained 5 μ L of RT-reaction, 200-nM primers, and 2X SYBR Green PCR Mastermix. DNA amplification cycles consisted of 1 cycle at 50°C for 2 min and 1 cycle at 95°C for 10 min, followed by a two step PCR procedure consisting of 15 sec at 95°C and 1 min at 60°C for 50 cycles. The amplification was performed using an ABI Prism 7500 Sequence Detector system (Applied Biosystems). The data were analyzed by SDS v 1.2 software (Applied Biosystems, Foster City, CA).

Results

Virological outcome in SHIV_{SF162P3}-infected rhesus macaques

Eight animals were challenged rectally with SHIV_{SF162P3} challenge stock. Plasma viral RNA load in each animal was measured over 140 wk, and the results are presented in Fig. 1A. All eight animals were readily infected as evidenced by high viral load at the peak of viremia (days 14–21, Fig. 1B). There was no correlation between virus challenge dose and viral load at peak viremia in this study. As expected, virus was also isolated from eight animals when PBMCs harvested on day 21 were co-cultured with PHA-activated human PBMCs (data not shown). When RNA load was measured post-peak viremia, it was observed that in six animals viremia was controlled below the detection limit of the assay (controllers). However, in two animals viremia at most time points during the chronic phase remained high and were easily detectable throughout the course of infection (non-controllers). For animal 713L viremia dropped to undetectable levels at week 52, but rebounded at week 62 and steadily increased until the animal was euthanized on week 140. Consistent with the plasma viremia, CD4⁺ T-cell counts in blood declined slightly in controllers during the acute phase of infection (Fig. 2B), but returned to normal at later times. In contrast, CD4⁺ T-cell counts in non-controllers gradually declined to less than 10% of the original level (Figs. 2A and B). Animals in the controller group remained healthy for more than 140 wk, whereas the non-

FIG. 1. SHIV_{SF162P3} RNA levels in plasma in SHIV_{SF162P3}-infected rhesus macaques. Eight animals were challenged rectally with 5000 (713L and 714L), 2500 (717L, 718L, 719L, and 720L), or 100 (68M and 69M) TCID₅₀ of SHIV_{SF162P3} stock. SHIV RNA (vRNA) levels were measured by nucleic acid sequence-based amplification (NASBA) assay, and shown as copies per milliliter of plasma. (A) vRNA levels of eight animals over the entire course of infection are shown except animals 719L and 68M, that were sacrificed before 80 wk. (B) The vRNA loads in the early time points are expanded. Arrows indicate sample collection time points for microarray analysis (dotted arrows indicate 718L and 720L animals, and solid black arrows indicate 713L and 714L animals). Non-controllers are underlined in the key.

controllers developed AIDS-like symptoms and were eventually euthanized.

To determine whether the control of viremia observed in the majority of SHIV_{SF162P3}-infected animals might correlate with the anti-SHIV immune response, neutralizing antibodies to the challenge virus were examined. All SHIV_{SF162P3}-infected animals seroconverted and had high titers, and anti-

Env and anti-Gag binding antibodies were detected in the serum during the course of infection (data not shown). Sera from these animals, collected at different time points following challenge, were assayed for neutralization of the challenge virus. As shown in Fig. 3, neutralizing antibodies were detected in some of the infected macaques after 14 wk of infection. Typically antibody response peaked around 28 wk and slowly declined with time. Interestingly, both animals that failed to control viremia had high titers of neutralizing antibody response to SHIV_{SF162P3}, with the peak titers be-

FIG. 2. CD3⁺/CD4⁺ T-cell counts in SHIV_{SF162P3}-infected rhesus macaques. Numbers of CD3⁺/CD4⁺ T cells were determined using a FACScan flow cytometer and CellQuest software, and are shown as CD3⁺/CD4⁺ T-cell numbers in PBMCs. (A) CD3⁺/CD4⁺ T-cell counts of controllers and non-controllers are plotted over the entire course of the study except animals 719L and 68M, that were sacrificed before 80 wk. (B) CD3⁺/CD4⁺ T-cell counts at the early time points are expanded in this plot. Non-controllers are underlined in the key.

FIG. 3. Neutralizing antibody titers induced by SHIV_{SF162P3} infection. Neutralization assay with sera from SHIV_{SF162P3}-infected animals against the challenge virus was performed in U373 cells expressing a reporter gene β -galactosidase under HIV-1 LTR promoter. The results are expressed as dilution of serum inhibiting 50% of infection in U373 cells. Eight animals were challenged rectally with 5000 (713L and 714L), 2500 (717L, 718L, 719L, and 720L), or 100 (68M and 69M) TCID₅₀ of SHIV_{SF162P3} stock. The neutralization titers of controllers (714L, 717L, 718L, 719L, 68M, and 69M) and non-controllers (713L and 720L) were measured.

tween 500 and 600. However, among controllers only two animals had peak titers greater than 200. Neutralization titers of the sera of two non-controllers (713L and 720L) and two controllers (714L and 718L) persisted, and the titers at weeks 61 and 140 were comparable to those observed at week 48 (data not shown).

Differential expression of cellular genes in the PBMCs of controller and non-controller animals

To investigate the possibility that innate immune mechanisms, mediated by the regulation of specific host factors, might be involved in control of viremia and disease progression, global cellular gene expression was measured by microarray analysis in the infected animals. Four MamuA*01-negative infected macaques were selected from the eight infected animals based on plasma viremia during the chronic phase of infection. The group of non-controllers (713L and 720L) had high plasma viremia levels (between 7.2 and 7.4 log₁₀ vRNA copies/mL) in the acute phase of infection (day 21), and high plasma viremia levels (between 5 and 5.5 log₁₀ vRNA copies/mL) in the chronic phase of infection (>2 y). In contrast, the controllers (714L and 718L) had high plasma viremia in the acute phase of infection (between 6.5 and 6.8 log₁₀ vRNA copies/mL), but cleared virus to an undetectable level in the chronic phase of infection.

The gene expression profiles of the PBMCs of controllers and non-controllers in the acute and chronic phases of in-

fection were compared. To determine variability in gene expression patterns among naïve animals, we examined the levels of rADAR, rIFI44, rSTAT1, and rGAPDH mRNA in five naïve animals by real-time RT-PCR. The standard deviation of Ct values among these naïve animals was ± 1 Ct, which represents less than twofold variation in mRNA levels (data not shown). Based on these results, pooled PBMCs from eight uninfected rhesus macaques were used as the naïve control for this study to minimize variability. Because the variability among uninfected animals was less than the differences that were detected between naïve and controller or non-controller animals, it is unlikely that the variability among naïve animals would have an impact on the conclusions of the study.

Microarray expression files were generated for each sample and analyzed with various computational tools. When the samples from the two different time points (21 d and 2 y post-infection) were analyzed separately, a relatively small number of differentially expressed genes were identified due to the high variability within the small sample size. Four genes were identified in the samples from 21 d post-infection and seven genes were identified in the samples at 2 y post-infection with p -value <0.05 by the LPE test (data not shown). Interestingly, all four genes identified in the 21-d post-infection samples were upregulated among non-controllers. When the data from the two time points were combined and analyzed using the paired LPE test, 59 genes with p -values <0.01 were identified. These genes are described below and the clustering heatmaps and annotation information are shown in Fig. 4 and Table 1.

Functional grouping indicated that genes involved in interferon responses were continuously highly expressed in non-controllers both in the acute and chronic phases of infection. The expression of these genes was only modestly elevated in the acute phase of infection in the controllers. These genes include interferon- α -inducible protein 44 (IFI44), interferon-inducible protein p27 (IFI27), 2'-5'-oligoadenylate synthetase-like (OASL), 2'-5'-oligoadenylate synthetase 1 (OAS1), indoleamine-pyrrole 2,3 dioxygenase (INDO), interferon-stimulated protein, 15 kDa (ISG15), guanylate binding protein 67 kDa (GBP1), and signal transducer and activator of transcription 1 (STAT1) (Fig. 4).

Suppression of genes of the S100 family, which mediate various biological functions *in vivo*, was observed in controllers. The S100 family consists of calcium binding proteins containing a helix-loop-helix Ca²⁺ binding motif (EF-hand). There are at least 20 members, and they are implicated in intracellular and extracellular regulatory activities (6,11). Ca²⁺-dependent signals are essential for differentiation, proliferation, and apoptotic cell death of immune cells. Our data indicated that in controllers, expression of S100A9 was decreased at day 21 (Fig. 4A). However, in non-controllers, expression of this gene was increased at day 21 and in the chronic phase of infection (Fig. 4C). Expression of S100P, which is also a member of this family of Ca²⁺-binding proteins, was decreased in controllers in the acute phase, but its expression was increased in non-controllers in the acute and chronic phase of infection (Fig. 4C).

Several signal transduction-related genes were differentially expressed in both controllers and non-controllers during the acute phase of infection. CD8 antigen, beta polypeptide 1 expression was decreased in non-controllers but not

A

FIG. 4. The clustering heatmap for differentially expressed genes between controllers (714L and 718L) and non-controllers (713L and 720L). (A) Differentially expressed genes at day 21 (B) Differentially expressed genes at year 2. (C) Differentially expressed genes at both day 21 and year 2.

in controllers. In contrast, expression of toll-like receptor 2 (TLR-2) and regulator of G-protein signaling 2 (RGS2) were decreased in controllers, but were elevated in non-controllers (Fig. 4C).

Discussion

Global cellular gene expression patterns associated with the control of viremia have not been extensively studied. In this study, distinct gene expression patterns in PBMCs were noted between SHIV_{SF162P3}-infected controller and non-controller animals. Overall, 59 genes were differentially expressed between controllers and non-controllers that were infected with SHIV_{SF162P3}. Many of the differentially ex-

pressed genes identified in this study have been implicated previously in the pathogenesis of HIV-1-infected humans and SIV-infected macaques (4,31). Viral loads may be determined by factors related to differences in gene expression profiles, or conversely, virus loads may affect gene expression profiles. It is notable that in the acute phase of infection both controllers and non-controllers had high virus loads, but different gene expression profiles.

It is probably most instructive to analyze the changes in gene expression profiles in the context of gene products that interact in functionally related networks. For example, type I interferons bind to specific cell-surface receptors that activate JAK-STAT signaling pathways, leading to the activation of diverse cellular genes. Our finding of significantly higher

B **FIG. 4.** (Continued).

levels of expression of interferon-induced genes in non-controllers in the acute phase of infection compared to controllers might serve as an early indicator of disease progression. This is consistent with results from other investigators, who also observed the robust induction of interferon-inducible genes, OAS1, OASL, and IFI27 in the acute phase of SIV infection, and in animals that rapidly progressed to disease (4,31). In addition, high levels of MX mRNA are associated with high vRNA levels in lymphoid tissues from chronically infected animals (1). Similar results have been reported using gene expression profiling in intestinal mucosal biopsies of chronically HIV-1 infected patients with high viral loads (27). Recent evidence also suggested that high expression of interferon-induced genes and antiviral cytokines are not sufficient to prevent SIV replication and dissemina-

tion in a nonhuman primate model (2). Together, these results suggest that certain interferon responses may in fact facilitate replication of SIV and HIV-1.

S100 is a multigene family of non-ubiquitous Ca^{2+} -modulated proteins, and some S100 proteins are involved in the regulation of inflammation (6). S100 proteins have been implicated in the regulation of protein phosphorylation, some enzyme activities, dynamics of cytoskeleton components, transcription factors, Ca^{2+} homeostasis, and cell proliferation and differentiation (6). It also has been shown that S100A8, S100A9, and S100A12 are highly expressed in the myeloid cell lineage and are found in the extracellular milieu during infection and inflammatory conditions (8,28). Our data suggest that decreased expression of S100A9 in early infection in controllers may be associated with de-

C

FIG. 4. (Continued).

creased virus replication. In contrast, increased expression of S100P in the non-controllers in both acute and chronic phases of infection is consistent with high viral load in these animals. Similarly high levels of S100A8, S100A9, and S100A12 in the serum of HIV-1-infected patients correlated with low CD4⁺ T-cell counts and disease progression (12,26). Besides host cellular factors, adaptive immune responses induced in these animals following infection might have also played a role in controlling viral infection. Our analyses of the neutralization of challenge virus by the sera from these infected animals collected at different time points clearly demonstrated that there was no correlation between neutralizing antibody response induced by infection and the control of viremia and eventual disease progression. Although the role played by cell-mediated immune responses

in controlling viral infection was not extensively analyzed here, results presented elsewhere (19,29,30) and from our preliminary data (data not shown), suggest that CD8⁺ central memory T-cell responses might also play a role in the containment of viral infection in controller animals.

Conclusion

SHIV_{SF162P3}-infected macaques have been used extensively as a model to study the pathobiology of HIV-1 in humans. We have examined differences between protective and non-protective host immune response by measuring global cellular gene expression profiles in SHIV-infected macaques. In this study, no correlation was observed between neutralizing antibody response following infection and the control

TABLE 1. LIST OF 59 GENES IDENTIFIED

<i>Probe set ID</i>	<i>Gene symbol</i>	<i>Gene title</i>	<i>Molecular function</i>
204351_at	S100	S100 calcium binding protein P	Magnesium ion binding, calcium ion binding, protein binding, calcium-dependent protein binding
205237_at	FCN1	Ficolin (collagen/fibrinogen domain containing) 1	Antigen binding, receptor binding, calcium ion binding, sugar binding
212636_at	QKI	Quaking homolog, KH domain RNA binding (mouse)	RNA binding
201739_at	SGK	Serum/glucocorticoid regulated kinase	Nucleotide binding, protein kinase activity, protein serine/threonine kinase activity, protein binding, ATP binding, kinase activity, transferase activity
202411_at	IFI27	Interferon, alpha-inducible protein 27	SH3/SH2 adaptor activity, protein binding
202609_at	EPS8	Epidermal growth factor receptor pathway substrate 8	RNA binding, ATP binding, transferase activity, nucleotidyltransferase activity
205552_s_at	OAS1	2',5'-Oligoadenylate synthetase 1, 40/46 kDa	Transcription factor activity, transcription coactivator activity, transcription corepressor activity, transcription regulator activity
206715_at	TFEC	Transcription factor EC	Signal transducer activity, protein binding, protein binding, peptidase activity, cysteine-type peptidase activity, caspase activator activity, hydrolase activity, caspase activity,
211367_s_at	CASP1	Caspase 1, apoptosis-related cysteine peptidase (interleukin 1, beta, convertase)	DNA binding, endopeptidase inhibitor activity, serine-type endopeptidase inhibitor activity, binding, protein binding
211404_s_at	APLP2	Amyloid beta (A4) precursor-like protein 2	Retinal dehydrogenase activity, aldehyde dehydrogenase (NAD) activity, Ras GTPase activator activity, androgen binding, oxidoreductase activity
214453_s_at	IFI44	interferon-induced protein 44	Catalytic activity, glutamate-ammonia ligase activity, ligase activity
215001_s_at	GLUL	Glutamate-ammonia ligase (glutamine synthetase)	Catalytic activity, glutamate-ammonia ligase activity, ligase activity
200648_s_at	GLUL	Glutamate-ammonia ligase (glutamine synthetase)	Actin binding, actin binding, calmodulin binding, tropomyosin binding, oxidoreductase activity, myosin transition metal ion binding
201616_s_at	CALD1	Caldesmon 1	Receptor activity
201647_s_at	SCARB2	Scavenger receptor class B, member 2	Protein kinase activity, protein kinase activity, protein kinase inhibitor activity, protein binding, ATP binding
201670_s_at	MARCKS	Myristoylated alanine-rich protein kinase C substrate	Nucleotide binding, GTPase activity, GTP binding, GTP binding
202241_at	TRIB1	Tribbles homolog 1 (<i>Drosophila</i>)	Signal transducer activity, GTPase activator activity, protein binding, calmodulin binding
202269_x_at	GBP1	Guanylate binding protein 1, interferon-inducible, 67 kDa	Signal transducer activity, calcium ion binding, calcium ion binding
202388_at	RGS2	Regulator of G-protein signaling 2, 24 kDa	DNA binding, DNA binding, transcription factor activity, transcription co-repressor activity, sequence-specific DNA binding, protein dimerization activity
203041_s_at	LAMP2	Lysosomal-associated membrane protein 2	
203535_at	S100A9	S100 calcium binding protein A9	
203574_at	NFIL3	Nuclear factor, interleukin 3 regulated	

(continued)

TABLE 1. LIST OF 59 GENES IDENTIFIED (CONT'D)

<i>Probe set ID</i>	<i>Gene symbol</i>	<i>Gene title</i>	<i>Molecular function</i>
203922_s_at	CYBB	Cytochrome b-245, beta polypeptide (chronic granulomatous disease)	Ion channel activity, voltage-gated ion channel activity, iron ion binding, oxidoreductase activity, metal ion binding FAD binding Receptor binding, cytokinase activity, chemokine activity, cAMP-dependent protein kinase regulator activity Basic amino acid permease activity, amino acid transporter activity
204533_at	CXCL10	Chemokine (C-X-C motif) ligand 10	
204588_s_at	SLC7A7	Solute carrier family 7 (cationic amino acid transporter, y ⁺ system), member 7	
204838_s_at	MLH3	MutL homolog 3 (<i>E. coli</i>)	
204860_s_at	LOC728519, NAIP	NLR family, apoptosis inhibitory protein similar to baculoviral IAP repeat-containing protein 1 (neuronal apoptosis inhibitory protein)	Satellite DNA binding, protein binding, ATP binding, mismatched DNA binding Nucleotide binding, ATP binding, nucleoside-triphosphatase activity
204924_at	TLR2	Toll-like receptor 2	
205483_s_at	ISG15	ISG15 ubiquitin-like modifier	Lipopolysaccharide receptor activity, receptor activity, transmembrane, receptor activity, protein binding, pattern recognition receptor activity, peptidoglycan binding, Protein binding
205660_at	OASL	2'-5'-Oligoadenylate synthetase-like	
205842_s_at	JAK2	Janus kinase 2 (a protein tyrosine kinase)	DNA binding, RNA binding, double-stranded RNA binding, ATP binding, transferase activity thyroid hormone receptor binding Nucleotide binding, protein kinase activity, protein-tyrosine kinase activity, Janus kinase activity, receptor binding, protein binding, ATP binding, kinase activity Transcription cofactor activity, receptor binding, ErbB-2 class receptor binding, growth factor activity, transmembrane receptor protein tyrosine kinase activator activity, receptor tyrosine kinase binding
206343_s_at	NRG1	Neuregulin 1	
206420_at	IGSF6	Immunoglobulin superfamily, member 6	
206710_s_at	EPB41L3	Erythrocyte membrane protein band 4.1-like 3	
207857_at	LILRA2	Leukocyte immunoglobulin-like receptor, subfamily A (with TM domain), member 2	Transmembrane receptor activity Actin binding, structural molecule activity, binding, protein binding, cytoskeletal protein binding Antigen binding, receptor activity, receptor activity
207979_s_at	CD8B	CD8b molecule	
209230_s_at	NUPR1	Nuclear protein 1	Protein binding, protein binding, coreceptor activity, coreceptor activity, MHC class I protein binding, MHC class I protein binding
209499_x_at	TNFSF12- TNFSF13	Tumor necrosis factor (ligand) superfamily, member 13 Tumor necrosis factor (ligand) superfamily, member 12-member 13	
209969_s_at	STAT1	Signal transducer and activator of transcription 1, 91 kDa	DNA binding, transcription factor activity, signal transducer activity, hematopoietin/interferon-class (D200-domain) cytokine receptor signal transducer activity, calcium ion binding protein

(continued)

TABLE 1. LIST OF 59 GENES IDENTIFIED (CONT'D)

<i>Probe set ID</i>	<i>Gene symbol</i>	<i>Gene title</i>	<i>Molecular function</i>
209970_x_at	CASP1	Caspase 1, apoptosis-related cysteine peptidase (interleukin 1, beta, convertase)	Signal transducer activity, protein binding, peptidase activity, cysteine-type peptidase activity, caspase activator activity, hydrolase activity, caspase activity
210029_at	INDO	Indoleamine-pyrrole 2,3 dioxygenase	Indoleamine-pyrrole 2,3-dioxygenase activity, iron ion binding electron carrier activity, oxidoreductase activity, acting on single donors with incorporation of molecular oxygen, incorporation of two atoms of oxygen
211368_s_at	CASP1	Caspase 1, apoptosis-related cysteine peptidase (interleukin 1, beta, convertase)	Signal transducer activity, protein binding, peptidase activity, cysteine-type peptidase activity, caspase activator activity, hydrolase activity, caspase activity
212192_at	KCTD12	Potassium channel tetramerization domain containing 12	Voltage-gated potassium channel activity, protein binding
212268_at	SERPINF	Serpin peptidase inhibitor, clade B (ovalbumin), member 1	Endopeptidase inhibitor activity, serine-type endopeptidase inhibitor activity
212289_at	ANKRD12	Ankyrin repeat domain 12	
212335_at	GNS	Glucosamine (N-acetyl)-6-sulfatase (Sanfilippo disease IIID)	Calcium ion binding, N-acetylglucosamine-6-sulfatase activity, N-acetylglucosamine-6-sulfatase activity, sulfuric ester hydrolase activity, hydrolase activity, metal ion binding
212820_at	DMXL2	Dmx-like 2	Translation initiation factor activity, Rab GTPase binding
213109_at	TNIK	TRAF2 and NCK interacting kinase	Nucleotide binding, protein kinase activity, protein serine/threonine kinase activity, small GTPase regulator activity, ATP binding
213516_at	AKAP13	A kinase (PRKA) anchor protein 13	Signal transducer activity, receptor activity, guanyl-nucleotide nucleotide exchange factor activity, Rho guanyl-nucleotide exchange factor activity, zinc ion binding, diacylglycerol binding, metal ion binding
214894_x_at	MACF1	Microtubule-actin cross-linking factor 1	Actin binding, hydrolase activity, hydrolyzing O-glycosyl compounds, calcium ion binding, protein binding, microtubule binding
215223_s_at	SOD2	Superoxide dismutase 2, mitochondrial	Superoxide dismutase activity, manganese superoxide dismutase activity, oxidoreductase activity, manganese ion binding, metal ion binding
215594_at	SOD2	PRT2 mRNA for repetitive sequence	
216841_s_at	SOD2	Superoxide dismutase 2, mitochondrial	
217340_at	LOC645452, LOC650644	Similar to 60S ribosomal protein L21	
217502_at	IFIT2	Interferon-induced protein with tetratricopeptide repeats 2	Superoxide dismutase activity, manganese superoxide dismutase activity, oxidoreductase activity, manganese ion binding, metal ion binding
218728_s_at	CNIH4	Connexin homolog 4 (<i>Drosophila</i>)	Structural constituent of ribosome
221225_at	DCAKD	Dephospho-CoA kinase domain containing	
222156_x_at	CCPG1	Cell cycle progression 1	Binding
			Protein binding
			ATP binding, kinase activity

of viremia and eventual disease progression. However, comparing gene expression profiles in peripheral blood mononuclear cells from animals that exhibit different levels of virus control showed interesting differences in gene expression patterns (Fig. 4). The results presented here suggest that global gene expression analysis may provide a complementary approach for analyzing the cell-mediated immune response, and could lead to a better understanding of the molecular determinants involved in innate immunity, and could help identify early parameters that predict the efficiency of protective immune responses.

Activation of host defense-related genes such as interferon-inducible genes, apoptosis genes, and inflammation-related genes (balance of cell homeostasis) might contribute to a favorable microenvironment for virus replication *in vivo*. The advantage of the global gene expression profiling approach, especially when applied to specific immune cell subsets, is that it offers the unique possibility of monitoring the earliest steps in the innate and adaptive immune responses. Ultimately, it will be useful to correlate cell function with gene expression profiles in specific types of immune cells. Obviously, this will require analysis of larger groups of animals.

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Disclosure Statement

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