

**Magnitude and quality of cytokine and chemokine storm during acute infection distinguish non-progressive and progressive simian immunodeficiency virus infections of nonhuman primates**

**Running title: Cytokine responses in acute SIV infection**

Sheila M. Keating,<sup>a,b,#</sup> John W. Heitman,<sup>a</sup> Shiquan Wu,<sup>a</sup> Xutao Deng,<sup>a</sup> Andrea R. Stacey,<sup>d1</sup> Roland C. Zahn,<sup>f,2</sup> Maurus de la Rosa,<sup>f,3</sup> Samantha L. Finstad,<sup>f,4</sup> Jeffrey D. Lifson,<sup>g</sup> Michael Piatak, Jr.,<sup>g,5</sup> Marie-Claire Gauduin,<sup>h</sup> Benedikt M. Kessler,<sup>d,e</sup> Nicola Ternette,<sup>d,e</sup> Angela Carville,<sup>i,6</sup> R. Paul Johnson,<sup>j,7</sup> Ronald C. Desrosiers,<sup>k,8</sup> Norman L. Letvin,<sup>f,5</sup> Persephone Borrow,<sup>d</sup> Philip J. Norris<sup>a,b,c,%</sup>, and Joern E. Schmitz<sup>f,9,%</sup>

<sup>a</sup>Blood Systems Research Institute, San Francisco, CA, USA, Departments of <sup>b</sup>Laboratory Medicine and <sup>c</sup>Medicine, University of California, San Francisco, CA, USA;

<sup>d</sup>Nuffield Department of Clinical Medicine and <sup>e</sup>Target Discovery Institute, University of Oxford, UK;

<sup>f</sup>Center for Virology and Vaccine Research, Beth Israel Deaconess Medical Center, Harvard University, Boston, MA, USA;

<sup>g</sup>AIDS and Cancer Virus Program, Leidos Biomedical Research, Inc., Frederick National Laboratory, Frederick, MD, USA;

<sup>h</sup>Texas Biomedical Research Institute, Department of Virology and Immunology and Southwest National Primate Research Center, San Antonio, Texas, USA;

Departments of <sup>i</sup>Primate Resources, <sup>j</sup>Immunology and <sup>k</sup>Microbiology, New England Primate Research Center, Southborough, MA, USA;

<sup>1</sup>Current address: Immunocore, Abingdon, UK

<sup>2</sup>Current address: Janssen Infectious Diseases and Vaccines, Leiden, The Netherlands

<sup>3</sup>Current address: Baxter Innovations GmbH, Wien, Austria

<sup>4</sup>Current address: National Institutes of Health, National Cancer Institute, Bethesda, USA

<sup>5</sup>deceased

<sup>6</sup>Current address: Biomedical Research Models, Worcester, USA

<sup>7</sup>Current address: Yerkes National Primate Research Center, Emory University, Atlanta, USA

<sup>8</sup>Current address: University of Miami Miller School of Medicine, Miami, USA

<sup>9</sup>Current address: Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Aachen, Germany

<sup>%</sup>PJN and JES contributed equally to this work.

<sup>#</sup>Address correspondence to Sheila M. Keating, Blood Systems Research Institute, 270 Masonic Avenue, San Francisco, CA 94127. Email: [skeating@bloodsystems.org](mailto:skeating@bloodsystems.org)

Word count for abstract: 232; Word count for text: 5101

## ABSTRACT

Acute HIV infection represents a period of intense immune perturbation and activation of the host immune system. Study of the eclipse and viral expansion phases of infection is difficult in humans, but studies in non-progressive and progressive nonhuman primate infection models can provide significant insight into critical events occurring during this time. Cytokines, chemokines and other soluble immune factors were measured in longitudinal samples from rhesus macaques infected with either SIVmac251 (progressive infection) or SIVmac239 $\Delta$ *nef* (attenuated / non-progressive infection), and from African green monkeys infected with SIVsab9315BR (non-pathogenic infection). Levels of acute-phase peak viral replication were highest in SIVmac251 infection, but correlated positively with viremia at three months post-infection in all three infection models. SIVmac251 infection was associated with stronger corresponding acute-phase cytokine/chemokine responses than the non-progressive infections. Production of IL-15, IL-18, IFN- $\gamma$ , G-CSF, MCP-1, MIP-1 $\beta$  and SAA during acute SIVmac251 infection, but not SIVmac239 $\Delta$ *nef* or SIVsab9315BR infection, correlated positively with chronic viremia at three months post infection. Acute-phase production of MCP-1 correlated with viremia at three months post infection in both non-progressive infections. Finally, a positive correlation between the acute-phase area under the curve (AUC) IL-6 and sCD40L and chronic viremia was only observed in the non-progressive infections models. While we observed dynamic acute inflammatory immune responses in both progressive and non-progressive SIV infections, the responses in the non-progressive infections were not only lower in magnitude but also qualitatively different biomarkers of disease progression.

69 **IMPORTANCE**

70

71 NHP models of HIV infection constitute a powerful tool to study viral pathogenesis to gain  
72 critical information for a better understanding of HIV infection in humans. Here, we studied  
73 progressive and non-progressive SIV infection models in both natural and non-natural host  
74 NHP species. Regardless of the pathogenicity of the virus infection or NHP species  
75 studied, the magnitude of viremia, as measured by area under the curve during the first 4  
76 weeks of infection, positively correlated with viremia in chronic infection. The magnitude of  
77 cytokine and chemokine responses during primary infection also correlated positively with  
78 both acute-phase and chronic viremia. However, the pattern and levels of specific  
79 cytokines and chemokines produced differed between non-progressive and progressive  
80 SIV infection models. The qualitative differences in the early immune response in  
81 progressive and non-progressive infections identified here correlate with and may provide  
82 insights into the basis of differences in the subsequent course of disease.

83

## INTRODUCTION

Acute HIV infection lasts only for a few weeks, but the dynamics of virus/host interactions during this period can have lasting consequences through the course of infection (1, 2). Understanding the early immune responses, including the identity, kinetics, and magnitude of production of soluble mediators of inflammation, may shed light on how these early responses can impact subsequent course of the infection.

Although the identification of infected humans during the eclipse period of HIV infection is extremely difficult (3), a study conducted on plasma donor panels effectively described the kinetics of systemic cytokine immune responses during the days to weeks following HIV transmission (4). Rapid elevations in cytokines including IFN- $\alpha$  and IL-15 were reported prior to peak viremia in acute infection, while slightly slower, more sustained elevations were observed in other analytes including IL-6, IL-8, IL-18, and IFN- $\gamma$  along with a later-peaking increase in IL-10. There was also a biphasic increase in acute phase proteins, including serum amyloid A (SAA), indicating an early response to infection followed by further acute-phase protein induction coinciding with production of pro-inflammatory cytokines TNF- $\alpha$ , IL-6 and IL-22 (5). While these studies give valuable insight into the chronology of immune induction during acute HIV infection, it is not known how these cytokine responses relate to the subsequent disease course as long term virologic and clinical follow up was not available for these individuals.

The potential functional effects of cytokines on the immune system and HIV replication are complex. Many cytokines are pleiotropic and can even have opposing effects, simultaneously promoting suppression of virus replication and enabling virus replication by immune activation (e.g., IL-15 or TNF- $\alpha$ ). Only very few cytokine responses

are thought to have an overall beneficial effect for the HIV-infected individual (reviewed in (6). Nonhuman primate (NHP) models of HIV infection enable study of the relationship between viral replication and cytokine responses under controlled experimental settings (7). These models include simian immunodeficiency virus (SIV) infection of Asian macaques that are non-natural hosts of SIV, such as SIVmac infection of Indian rhesus macaques (RM) and infection of species that are endemic natural hosts of SIVs, such as SIVagm infection of African green monkeys (AGM) (8). The course of experimental SIV infection is variable in RM and depends on the particular virus strain used for infection. Similar to untreated HIV infection in humans, experimental SIVmac251 infection of RM is highly pathogenic, resulting in a rapid increase in viremia followed by partial containment but ongoing viral replication. It is associated with pathological immune activation, progressive decline of CD4<sup>+</sup> T cell populations and eventually there is a complete loss of viral containment with development of clinically apparent immunodeficiency manifested by AIDS defining conditions including opportunistic infections, wasting and death (9). In contrast, infections of RM with live attenuated viruses such as SIVmac239Δ*nef* are generally much less pathogenic (10). Viremia is more efficiently contained resulting in a very low chronic phase viremia that is maintained either indefinitely or for prolonged periods of time. In contrast to SIV infections of non-natural hosts, SIV infections in natural NHP hosts such as SIVagm infection of AGM do not typically result in serious disease (8). Although chronic phase viral replication and viremia are only partially contained, the loss of CD4<sup>+</sup> T cells is transient, immune activation is limited to primary infection, and progression to AIDS does not occur.

Progressive SIV infection in RM results in increased expression of markers for cell activation and proliferation along with sustained production of cytokines, including type I

IFN (11, 12). Heightened T cell turnover occurs in response to viral infection and, in the presence of persistent pro-inflammatory cytokines, results in loss of memory T cells (13, 14). A number of similarities between non-progressive and progressive SIV infections have been identified, including an initial type I IFN response accompanying the increase in acute viremia (14). However, natural hosts are able to down-modulate the virus-associated immune activation, including resolution of type I IFN up-regulation. Natural hosts are also able to maintain functional mucosal immunity including preservation of T regulatory and Th17 cells, maintenance of gut mucosal integrity with no increased microbial translocation, and limited systemic immune activation in chronic infection (15). A comparison of transcriptional profiles of non-progressive and progressive SIV infections has revealed differences in transcriptional kinetics in lymphoid tissues (16). The gene expression patterns in progressive infection exhibit a shift toward general Th1 immune responses with strong and sustained IFN type I and II responses, loss of T regulatory cells and loss of control of T cell activation (17-19). Although the magnitude of systemic immune activation at the time of acute infection is comparable between non-progressive and progressive SIV infections at the gene expression level, cytokine/chemokine protein levels have not been extensively tested (16).

Although individual cytokine signals have been previously measured in early SIV infection, the ability to measure multiple protein analytes simultaneously and sensitively by cytokine bead multiplex assay has allowed a more comprehensive approach to the study of inflammatory mediators in acute viral infections (4, 20-22). Cytokine multiplex assays have been developed using antibodies against human cytokines that were found to be cross-reactive against NHP analytes. Here, we investigated the magnitude of changes in plasma levels of 25 analytes following progressive infection of RM with SIVmac251 and non-

156 progressive infections of RM with SIVmac239 $\Delta$ *nef* and AGM with SIVsab9315BR. Our  
157 analysis reveals differences in acute phase levels of several cytokines in non-progressive  
158 and progressive SIV infections and shows which patterns of cytokine production in acute  
159 infection correlate with chronic phase viremia.

160

## MATERIALS AND METHODS

### Animals and Viruses

A total of 19 sabaeus AGM (*Chlorocebus sabaeus*) were studied for baseline cytokine/chemokine levels. The animals were either imported from St. Kitts in the Caribbean or were purchased from the New Iberia Research Center, Louisiana. Six of these sabaeus AGM were studied after infection with an equivalent of 143ng p27 tissue culture supernatant of Molt4(cl8) cells infected with SIVsab9315BR. Cell-free virus was originally isolated from cerebrospinal fluid (CSF) and homogenized brain and LN tissues of AGM 9315 at the time of necropsy(23). All AGM were maintained in accordance with the guidelines of the Committee on the Care and Use of Laboratory Animals under an NIAID-approved animal study protocol, and all studies and procedures were reviewed and approved by the Institutional Animal Care and Use Committees of the NIH and Harvard University. The 6 SIVsab9315BR-infected AGM were part of the control group for a study to compare B and T cell depletion during acute infection (24). These animals received intravenous 50 mg/kg IgG (Gammaguard®, provided as IgIV; NIH Nonhuman Primate Reagent Resource) on the day of infection. The AGM infected with SIVsab9315BR were bled prior to infection and at multiple time points throughout the course of acute and chronic infection.

A total of 74 RM were studied for baseline cytokine and chemokine calculations. Ten RM were infected with SIVmac251 by intravenous (i.v.) route at a dose equivalent of 0.15 ng SIV p27 Gag per RM. Ten additional RM were infected with SIVmac239 $\Delta$ *nef* by i.v. route at a dose equivalent of 50 ng SIV p27 Gag per animal. The infected RM were bled



prior to infection and at multiple time points throughout the course of acute and chronic infection. The SIVmac251-infected RM animals received monoclonal IV IgG one week before infection (50 mg human immunoglobulin IgIV; NIH Nonhuman Primate Reagent Resource), as these animals were part of control groups in experimental studies (J.E. Schmitz; unpublished observations). The SIVmac239 $\Delta$ *nef*-infected RM did not receive an antibody injection until to the end of the observation period described here. All RM were maintained in accordance with the guidance of the Standing Committee on Animals for the Harvard Medical School and the Guide for the Care and Use of Laboratory Animals (25). Although the SIVmac239 $\Delta$ *nef*-infected RM involved in this study were maintained with standard research and veterinary practices, additional aspects of the SIVmac239 $\Delta$ *nef*-experiment that are not pertinent for this manuscript were not included in the protocol and, thus, were not contemporaneously approved by the HMS IACUC. When the investigators and the IACUC realized this protocol error, the IACUC determined that the unapproved procedures in question in all likelihood would have been approved if they had been described in the protocol.

## **Cell Stimulation**

Peripheral blood mononuclear cells (PBMC) from HIV-negative humans (n=8), SIV-negative RM (n=11) and AGM (n=6) were cultured in RPMI with 10% FCS alone or with phytohemagglutinin M (Sigma, Atlanta, GA) (PHA-M, 5 $\mu$ g/mL), lipopolysaccharide (Sigma) (LPS, 1ng/mL), phorbol 12-myristate 13-acetate (Sigma) (PMA, 10ng/mL)/Ionomycin (Sigma) (calcium ionophore; 1 $\mu$ g/mL) or the imidazoquinoline TLR7/8 agonist CLO-97 (Invitrogen, San Diego, CA) (2.5  $\mu$ g/mL). The supernatants from unstimulated, PHA-M, LPS, and PMA/Ionomycin stimulated cells were harvested after 24 hours of incubation, and

208 CLO-97 stimulated cells after 18 hours. The human samples were collected with informed  
209 consent under a protocol approved by the University of California, San Francisco (UCSF)  
210 Committee on Human Research.

211

## 212 **Determination of plasma virus levels**

213 Plasma virus levels of SIVmac251 and SIVmac239 $\Delta$ *nef* were determined by a real-time  
214 reverse transcription-PCR assay, essentially as described previously (Quantitative  
215 Molecular Diagnostic Section, AIDS Vaccine Program, NCI-Frederick, Frederick, MD; limit  
216 of detection as used in the present studies, 60 RNA copies/ml) (26). SIVsab9315BR  
217 plasma RNA levels in sabaeus AGM were quantified by the Ultrasense One-Step  
218 Quantitative RT-PCR System (Invitrogen Corp., Carlsbad, CA), as described previously  
219 (24).

220

## 221 **Cytokine/chemokine Analysis**

222 Twenty-three cytokines/chemokines were measured using the nonhuman primate cytokine  
223 Milliplex kit (Millipore, Billerica, MA) according to the manufacturer's instructions. This panel  
224 measured IL-1 $\beta$ , IL-1 receptor antagonist (ra), IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12/23  
225 (p40), IL-13, IL-15, IL-17, IL-18, IFN- $\gamma$ , G-CSF, GM-CSF, MCP-1, MIP-1 $\alpha$ , MIP-1 $\beta$ , TNF- $\alpha$ ,  
226 TGF- $\alpha$ , sCD40L, and VEGF. The analyte detection ranged from 0.64 to 10,000 pg/mL.  
227 Plasma or PBMC supernatant was incubated overnight with antibody-coupled beads  
228 followed by incubation with biotinylated detection antibody, and finally, incubation with  
229 streptavidin-PE. Each sample was assayed in duplicate, and cytokine standards and  
230 controls supplied by the manufacturer were run on each plate. Multi-analyte profiling was  
231 performed using a Luminex-100 system; data were analyzed using Bio-Plex manager

software (Bio-Rad, Hercules, CA). Analytes determined to be out of range below the standard by Bio-Plex were assigned values of half the difference between the lowest standard and zero. Human IFN- $\alpha$  was measured by ELISA (PBL Interferon Source, New Jersey) detecting 11 of the 14 known types of IFN- $\alpha$ . Levels of SAA were measured using a colorimetric ELISA (Multispecies SAA ELISA kit, Tridelta Development Ltd., County Kildare, Ireland). Diluted plasma samples were assayed in duplicate; concentrations were calculated from the standard curve obtained with a mixture of standards from different species such as bovine, porcine, canine, feline and equine provided with the kit.

## **Statistical Analysis**

In the validation experiment, supernatants from PBMC that were stimulated in culture were compared for differences in analyte levels by Kruskal Wallis ANOVA with Dunn's multiple comparison tests. Increased expression of cytokines/chemokines after infection was identified by establishing a threshold of reactivity. To determine this threshold, baseline plasma levels of all analytes were measured in uninfected animals; the upper 95% confidence limit of the mean analyte value for uninfected animals was determined. This criterion, in addition to two-fold baseline value and greater than 5 pg/mL, were used to establish the threshold of response. In the cytokine/chemokine comparative analysis, only analytes that were elevated in 50% or more animals per group were considered positive for the group. In individual cytokine/chemokine analyses, comparisons were made between analyte and viremia for each animal. Analyte levels were compared between two groups by Mann-Whitney test or between multiple groups by one-way ANOVA followed by Tukey Honestly Significant Differences (HSD). Linear regression was performed on all animals and cytokines by Spearman test to compare peak plasma viremia or area under the curve

256 (AUC) for the first 4 weeks with chronic day 98 or 100 viremia (grouped as three months).  
257 Linear regression by Spearman test was also performed to compare analyte (AUC or peak)  
258 concentration over the first four weeks with viremia in chronic infection. Throughout  
259 analyses, *P*-values were computed and then adjusted into FDR (False Discovery Rates) by  
260 the Benjamini and Hochberg controlling procedure (27). Statistical significance was  
261 indicated by *P* value <0.05 and FDR <0.1. The statistical software R/Bioconductor (Version  
262 2.15.1 with gdata, gee, ggplot2, grid, lattice, lme4, multtest, npmc, outliers, pwr, stats) and  
263 GraphPad Prism version 6.0 were utilized for analyses.

264

265

266

## RESULTS

### Recognition of RM and AGM analytes by cytokine/chemokine assays

To evaluate the consistency of recognition of each analyte across primate species in the assays, we first validated the assays. As gold standard primate-specific cytokines are currently not available, we performed an in-house validation of the assays using supernatants from human and NHP PBMC stimulated *in vitro* with compounds that elicit a broad spectrum of cytokines/chemokines. We assessed the ability of the Millipore 23-plex NHP cytokine panel and a human IFN- $\alpha$  ELISA to recognize RM and AGM cytokines and chemokines. Assay validation using supernatants from two PBMC samples from each of seven different primate species stimulated with LPS and PHA-M to signal through TLR-4 and CD3, respectively, had been previously performed by Millipore according to the user guide provided for this assay. The current validation expanded on the prior work and included the additional stimulants PMA/Ionomycin and CLO-97 to stimulate via protein kinase C and TLR-7/8, respectively, to maximize the diversity of cytokines/chemokines produced. The Milliplex NHP panel identified elevations in every analyte in response to at least one of the stimuli employed in the supernatants from human PBMCs; for the vast majority of the analytes this was also the case in both RM and AGM (**Table 1**). The heat map summarizes the responses across the primate species and the stimulants (**Figure 1**). Although the Luminex and ELISA assays were able to detect most cytokines and chemokines in the panel in NHP species and humans, *in vitro* stimulation of PBMC did not lead to detection of a significant increase over the unstimulated controls in levels of some analytes in one or both of the NHP species. Measurements of IL-10, IL-15, MCP-1, and TGF- $\alpha$  in supernatants from *in vitro* activated PBMC from AGM did not show any significant

increase. In RM, all chemokines/cytokines except for IL-15 (Luminex) and IFN- $\alpha$  (ELISA) rose significantly after *in vitro* stimulation of PBMC compared to unstimulated controls, although lack of statistically significant differences from baseline was due to only two samples being available for testing. Failure to detect elevations in a particular analyte in this experiment may indicate that the analyte was not detected by the assay; but could also indicate that the analyte was not produced after *in vitro* PBMC stimulation in the species concerned. Notably, some of the analytes in which elevations were not detected following *in vitro* stimulation of PBMCs were subsequently detected in NHP plasma samples, showing that the assay was able to detect them, but suggesting that they were not substantially up-regulated after *in vitro* stimulation; this was observed for MCP-1, IL-10 and IL-15, possibly due to the fact that the *in vitro* stimulation conditions selected did not reflect *in vivo* immune induction. As the majority of cytokines and chemokines examined were detected in both RM and AGM, we included all analytes in our subsequent analysis. SAA was not evaluated in the validation experiments. To minimize species related variation in the detection of SAA levels, a multi-species based ELISA assay was used in which the data were normalized against a standard mixture containing SAA derived from multiple species.

#### **Comparison of viral dynamics in SIVmac251- and SIVmac239 $\Delta$ *nef*-infected RM and SIVsab9315BR-infected AGM**

Previous investigations have shown a significant correlation between magnitude of peak viremia and viremia at three months post-infection of SIVmac251-infected RM (28); similarly, our data also indicated a significant correlation ( $P=0.03$ ). Notably, we observed that the first 4-week AUC viremia and three months post-infection in SIVmac251-infected

RM were significantly correlated ( $P = 0.03$ ; **Figure 2A and B**). A comparison of RM infected with SIVmac251 to RM infected with SIVmac239 $\Delta$ *nef* or SIVsab9315BR-infected AGM revealed a similar dynamic response with peak viremia at 2 weeks followed by a steady decline to a setpoint viremia in the non-progressive infections, although both non-progressive infections were characterized by somewhat lower median peak viremia and viremia at three months post-infection than the pathogenic infection (**Figure 2A**). Despite the non-pathogenic nature of SIVsab9315BR in AGM, a significant correlation was observed between the first 4-week AUC and viremia at three months post-infection in SIVsab9315BR-infected AGM ( $P=0.03$ ; **Figure 2A and B**). In contrast to the other two infections, there was not a significant correlation between the first 4-week AUC and viremia at three months post-infection in SIVmac239 $\Delta$ *nef* infected RM, but potentially a trend for a positive correlation ( $P=0.16$ ; **Figure 2A and B**).

### **Elevations in plasma levels of more cytokines and chemokines are detected after progressive SIV infection**

To determine the baseline plasma levels of our cytokine/chemokine, we measured plasma analyte levels in 74 RM and 19 AGM that were all SIV negative. Elevations in each analyte above the species baseline were then evaluated over time in each infection model. Of the 25 analytes tested, 19, 5 and 13 analytes were elevated in SIVmac251-infected RM, SIVmac239 $\Delta$ *nef*-infected RM, and SIVsab9315BR-infected AGM at any time point, respectively. More analytes showed detectable elevations in SIVmac251-infected RM compared to SIVmac239 $\Delta$ *nef*-infected RM or SIVsab9315BR-infected AGM (**Table 2**,  $P<0.0001$  and  $P=0.004$ , respectively). The cytokines and chemokines exhibiting an elevation in each animal model at serial time points after infection are shown in **Figure 3**.

Of note, the analytes that were elevated in both SIVmac251- and SIVsab9315BR-infected animals were G-CSF, IFN- $\gamma$ , IL-1 $\beta$ , IL-1ra, IL-6, IL-8, IL-10, IL-15, MCP-1, MIP-1 $\alpha$ , sCD40L and IFN- $\alpha$ . GM-CSF, IL-8, IL-15, MCP-1 and sCD40L were elevated in SIVmac239 $\Delta$ *nef* infection and IL-2, MIP-1 $\beta$ , TNF- $\alpha$ , VEGF, and IL-18 were uniquely recognized in SIVmac251 while IL-5 was only recognized in SIVsab9315BR infection.

### **Many cytokines were not elevated in acute SIV infection**

A number of analytes never crossed the threshold used to define a positive response during the course of acute infection. If fewer than 50% animals exhibited a positive response for a given analyte, up-regulation of that analyte was determined to be negative for the group. Increases were not detected for 6 of 25 analytes (IL-4, IL-5, IL-12, IL-13, IL-17, and TGF- $\alpha$ ) following SIVmac251 infection of RM, 20 of 25 analytes (G-CSF, IFN- $\gamma$ , IL-1 $\beta$ , IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, IL-13, IL-17, MIP-1 $\beta$ , MIP-1 $\alpha$ , TGF- $\alpha$ , TNF- $\alpha$ , VEGF, IL-18, IFN- $\alpha$ , and SAA) following SIVmac239 $\Delta$ *nef* infection of RM, and 11 of 25 analytes (GM-CSF, IL-2, IL-4, IL-12, IL-13, IL-17, MIP-1 $\beta$ , TGF- $\alpha$ , TNF- $\alpha$ , VEGF, and IL-18) following SIVsab9315BR infection of AGM. Regardless of NHP species or virus infection, we did not detect significant elevations in IL-4, IL-12, IL-13, IL-17 and TGF- $\alpha$ . Of note, each of these analytes except for TGF- $\alpha$  was detectable in both NHP species in our assay validation work (**Table 1**), demonstrating that apart from TGF- $\alpha$  the lack of detectable elevation was not due to the inability of the assay to detect the analyte. MIP-1 $\beta$ , VEGF and IL-18 did not increase after SIVsab9315BR infection of AGM and SIVmac239 $\Delta$ *nef* infection of RM. The only cytokines that were not detected in the validation studies and not detectably increased in SIV-infected NHP were GM-CSF and TGF- $\alpha$  in AGM.



### Peak cytokine responses are of higher magnitude after SIVmac251 infection

In addition to differences in the breadth of responses detected, progressive vs. non-progressive infections might differ in peak magnitude or timing of responses. To compare peak analyte levels, we focused on factors that were up-regulated after infection and did not significantly differ between the groups at baseline. Analyte levels in the SIVmac251- and SIVmac239 $\Delta$ *nef*-infected RM groups were not significantly different at baseline (**Figure 4**). However, IL-5 and MCP-1 were expressed at significantly higher baseline levels while IL-8 and IL-15 were expressed at significantly lower baseline levels in AGM compared to RM, and were thus excluded from comparative analysis. We first compared analyte increases in RM SIVmac251 infection and AGM SIVsab9315BR infection. Of the factors that were elevated in both comparison groups (**Table 3**), we found higher peak levels of IFN- $\gamma$ , IL-1 $\beta$ , IL-1ra, IL-5, IL-8, IL-10, MIP-1 $\alpha$  and IFN- $\alpha$  after SIVmac251 infection compared to SIVsab9315BR infection. SAA, IL-2, and IL-6 did not significantly differ at their peak response. G-CSF, GM-CSF, IL-2, IL-4, IL-12, IL-13, IL-17, MIP-1 $\beta$ , TGF- $\alpha$ , TNF- $\alpha$ , VEGF, IL-18 and sCD40L were not compared due to low or no responses in SIVsab9315BR- and/or RM SIVmac251-infected animals. Of the factors elevated in RM, GM-CSF, IFN- $\gamma$ , IL-1 $\beta$ , IL-1ra, IL-5, IL-6, IL-10, IL-15, MCP-1, MIP-1  $\alpha$ , MIP-1 $\beta$ , TNF-  $\alpha$ , VEGF, IFN- $\alpha$  and SAA peak levels were higher in SIVmac251-infected RM. Interestingly, a higher peak concentration of IL-8 in SIVmac239 $\Delta$ *nef*-infected animals occurred at day 56, after the peak of most other cytokine responses. Finally, sCD40L was higher in SIVsab9315BR than in SIVmac239 $\Delta$ *nef* infection. Within and across NHP species, peak levels of a number of analytes showing elevated levels were notably higher in the progressive than in non-progressive infections, attesting to the robustness of this observation.

## **Relationship between the peak viremia and peak cytokine responses during acute infection**

We hypothesized that acute viral replication could be associated with the strongest cytokine responses, and so we examined the correlation between peak cytokine levels and peak viremia during acute infection. No significant correlations were observed between peak cytokine responses and peak viremia in SIVmac251- and SIVmac239 $\Delta$ *nef*-infected RM (data not shown). In contrast, SIVsab9315BR infection of AGM was associated with a significant correlation between peak MCP-1 responses and peak viremia ( $P=0.006$ ). To determine the relationship between the general cytokine response over the first 4 weeks and peak viremia, we performed a correlation analysis between the cytokine 4-week AUC and peak viremia. We found a significant positive correlation in SIVmac239 $\Delta$ *nef*-infected animals for IL-8 ( $P=0.04$ ), a trend for positive correlation for MCP-1 ( $P=0.06$ ), and a trend for a negative correlation for GM-CSF ( $P=0.05$ ). However, this analysis did not result in significant correlations in the SIVmac251 or SIVsab9315BR-infected animals.

## **Early cytokine responses correlate with chronic viremia at three months**

Early cytokine responses were analyzed to identify a potential association with chronic viremia. The magnitude of the AUC cytokine responses of each individual animal over the first 4 weeks of infection was compared with the magnitude of chronic viremia at three months (**Table 4**). A number of individual cytokine responses in SIVmac251-infected RM significantly correlated with chronic viremia, including G-CSF, IFN- $\gamma$ , IL-15, MIP-1 $\beta$  and IL-18. In contrast, in the same analysis MCP-1 and sCD40L showed a significant correlation

411 in SIVmac239 $\Delta$ *nef*-infected RM, and IL-6 and MCP-1 in AGM infected with SIVsab9315BR.  
412 Additionally, a number of other cytokine responses during acute SIV infection were  
413 associated with viremia. A trend (*P* values between 0.05 and 0.2) was detected for the  
414 following analytes: sCD40L, IL-1 $\beta$  and IL-8 in SIVsab9315BR-infected AGM; and VEGF, IL-  
415 10, IL-6, and MIP-1 $\alpha$  in SIVmac251-infected RM (**Table 4**). Of note, all correlations  
416 detected were positive, i.e. a relatively higher magnitude of cytokine responses during early  
417 infection correlated with a higher magnitude of viremia at three months.

418

419

## DISCUSSION

Cytokines/chemokines were used as biomarkers of the systemic immune response to investigate their association with establishing virus/host balance. We used a cross-species application of human- and primate-specific ELISA and multiplex assays to study the induction of soluble immune mediators in acute and early SIV infection in models that do (SIVmac251-infected RM) or do not (SIVsab9315BR-infected AGM or SIVmac239 $\Delta$ *nef*-infected RM) typically show progressive disease leading to AIDS. A significantly higher magnitude and breadth of up-regulation of soluble immune factors was detected in RM infected with SIVmac251 compared to RM infected with SIVmac239 $\Delta$ *nef* or AGM infected with SIVsab9315BR. In all three SIV infection models, the magnitude of peak viremia did not correlate with the levels of soluble immune markers investigated during the first 4 weeks after infection. However, we observed in all three infection models that chronic viremia was correlated with the levels of several soluble immune markers detected during the first four weeks of infection. A predominantly pro-inflammatory signature profile was observed in progressive infection; this was in contrast to the monocyte chemotactic cytokine signature detected in the non-progressive SIV infection models.

During this study, all AGM were infused with 50mg/kg of IV IgG on the same day of SIV infection and the RM-challenged with SIVmac251 received 50mg/kg of IV IgG one week prior infection. Clinical studies in humans have found modest cytokine changes to occur within hours to days after IV IgG infusion, but have used an order of a magnitude higher doses (400-600 mg/kg) than were administered here (29). In a study of HIV-infected individuals, TNF- $\alpha$  was found to decline by 5 days after IV IgG infusion (30). In our study, we found no significant difference in the cytokine profile in pre-infection cytokines before and after IV IgG infusion for all cytokines determined here. Despite IV IgG infusion,

444 cytokine responses increased after infection in parallel with viremia and had similar kinetics  
445 between animal groups, albeit slightly delayed in the AGM. A similar post-infection cytokine  
446 peak has also been seen in other studies in RM that did not receive IV IgG (20). In our  
447 study, the SIV-induced peak of cytokine response is substantially longer than the timing of  
448 cytokine changes after IV IgG infusion in previous studies in humans (31). Taken together,  
449 there is no evidence that IV IgG administration to AGM or RM had any confounding effect,  
450 likely due to the significantly lower dose of IV IgG administered here.

451         For each animal, only a single pre-infection time point was available, which provided  
452 only limited information about the steady state cytokine/chemokine levels for baseline  
453 determination. This was overcome by measuring analytes in a large number of uninfected  
454 animals, and using the data to establish a baseline analyte level for each animal species.  
455 Before comparing the different infection models in two NHP species, we needed to confirm  
456 that the assays could detect these analytes across species. During the validation study, we  
457 established assay detection efficacy and found that the majority of the detection reagents  
458 were species cross-reactive. Nevertheless, it was difficult to determine if antibody-analyte  
459 binding was better in one species than another or whether variations in results were due to  
460 differences in levels of analyte production and/or re-absorption after stimulation of PBMC.  
461 This reveals a need for species-specific monoclonal antibodies and cytokine standards to  
462 be made to correctly quantify analyte concentrations for each species. Several analytes  
463 that showed better detection in the validation study in AGM were in fact expressed at  
464 higher levels in RM after SIV infection, including IL-5, IL-8, IL-1ra, IL-15, IL-18 and IFN- $\alpha$ ,  
465 which strongly suggests that these analytes were indeed produced in greater quantities  
466 during pathogenic infection.

As expected, the dynamics of the three viral infections were similar, while the progressive SIVmac251 infection model resulted in higher peak and chronic viremia than the two non-progressive infection models. As reported previously, SIVmac251 peak viremia was correlated with chronic viremia (28). This was also true for the non-progressive SIV infection in AGM. While not statistically significant, our data suggested a trend for a positive correlation between peak viremia and three months viremia in SIVmac239 $\Delta$ *nef*-infected RM. Thus, the magnitude of primary viremia appears to be associated with the magnitude of chronic viremia, regardless of the relative pathogenicity of SIV or the NHP species investigated.

Although we hypothesized that the inflammatory cytokine response would coincide and correlate with peak viremia, we did not find this to be true in RM and found that only in MCP-1 correlated SIVsab9315BR peak viremia in AGM ( $P=0.006$ ). In addition, peak viremia of SIVmac239 $\Delta$ *nef* showed weak and borderline positive correlations with the 4 week AUC for MCP-1 and IL-8 and negative correlation with 4 week AUC for GM-CSF. One of the main objectives of this study was to determine if any acute cytokine profiles were associated with chronic levels of viral replication. Comparing 4-week AUC cytokine levels in acute infection with setpoint levels of persisting viremia, we found that inflammatory cytokines IL-15, IL-18 and IFN- $\gamma$  positively correlated with viral setpoint in RM SIVmac251 infection. IL-15 and IL-18 promote activation of innate subsets and adaptive responses indirectly enhancing virus control while simultaneously driving CD4<sup>+</sup> T cell activation that promote stimulation and expansion of memory subsets of CD4<sup>+</sup> T cells providing potential reservoirs for viral expansion (32, 33); this may explain their positive correlation with chronic viremia. IFN- $\gamma$  is a key component in the maturation of the T cell response during acute infection and is associated with the activation of CD8<sup>+</sup> T cells and

the anti-viral NK cell response (34, 35). Several studies have shown that higher levels of IFN- $\gamma$  secreting antigen-specific T cells were associated with better control of SIV infection in RM (36), and IFN- $\gamma$  may be important in enhancing the expansion of a memory populations of immune cells for aiding long-term viral control in HIV infection (32). Given these potentially beneficial effects, the positive correlation of IFN- $\gamma$  with viral setpoint was unexpected. In humans, IFN- $\gamma$ , IL-12p40 and IL-12p70 responses were associated with a lower viral set point, while IL-7 and IL-15 were associated with higher setpoint (32, 36).

The only analyte showing increases during in the acute phase of infection and correlation with setpoint viremia in all three SIV-infection models was MCP-1, a marker of systemic monocyte macrophage trafficking. When the virus targets the CNS within days of infection, SIV-infected astrocytes produce the chemokine MCP-1 that is responsible for recruiting monocytes in SIV neuroinvasion and establishing the infected SIV reservoir in the brain (37). Suppressing monocyte activation in an acute infection model would be an attractive target in determining the relationship between monocyte activation and subsequent viral setpoint.

Another inflammatory cytokine, IL-6, uniquely correlated with viral setpoint in AGM, while sCD40L uniquely correlated with viral setpoint in SIVmac239 $\Delta$ *nef*-infected RM. Interestingly, IL-6 production is associated with severe pathogenicity in HIV infection in humans and SIV infection in RM (38-41). For example, SIVsmmPBj8 infection of RM results in death within a few weeks and induces significant IL-6 production which may be a cause or effect of the pathogenic course of infection (42). It is currently unknown why IL-6 production in AGM does not lead to pathogenicity. One can only speculate whether IL-6 production may in fact be beneficial for AGM. Given its pleiotropic effects, IL-6 may be

involved in the induction of regulatory B cells and thus restrain excessive immune activation, as recently described by Rosser *et al.* (43).

Acute SIV infection in both natural and non-natural hosts elicits a massive depletion of mucosal CD4<sup>+</sup> T cells. There is only a transient increase in inflammatory responses coinciding with acute viremia and eventually CD4<sup>+</sup> T cell levels return to normal (44). Although non-progressive SIV infection does not exhibit the same mucosal inflammation as progressive infection, recent work has shown that this non-progressive equilibrium can be disrupted in AGM using an experimental model of colitis resulting in gut-associated microbial translocation, macrophage accumulation, immune activation, significantly elevated viral load and continued decline of CD4<sup>+</sup> T cells, similar to progressive infection (45, 46). Treatment with IL-21 has been shown to reduce inflammation and support maintenance of mucosal Th17 cells preventing microbial translocation and inflammation in progressive infection (47). The initial inflammatory responses in both groups and the differences in outcome could be related to how the infection seeds at the mucosa and the immune responses present that induce or prevent immune dysfunction; this may be an innate immune difference in the animal species. Monocyte chemotactic factors (MCP-1 and IL-8) and inflammatory cytokine production (i.e. IL-1 $\beta$ , IL-6, TNF- $\alpha$  and IL-18) in progressive infections could stem from this initial inflammatory cascade at the mucosal level; investigation into the mucosal immune response of non-progressive hosts may shed light on some of these differences.

In summary, we observed higher levels of virus replication associated with a higher-magnitude of cytokine/chemokine response in progressive infection, and hypothesize that excessive early cytokine release may contribute to further immune activation and virus replication, setting the stage for subsequent disease progression. However, since we only



performed correlation analyses of the magnitude of cytokine responses and viremia in the three SIV infection models, the mechanistic basis for the difference between progressive and non-progressive disease course still remains elusive. In the future, interventional in vivo studies will have to be performed to determine whether the qualitative and quantitative differences in cytokine/chemokine responses contribute to the non-pathogenic nature in non-progressive SIV infection.

## **Acknowledgments**

This work was supported by NIH, NIAID, Division of AIDS, grant AI0678501 (CHAVI, Center for HIV AIDS Vaccine Immunology) (B.M.K., P.B., N.L.L., P.J.N., J.E.S.) and AI065335 (J.E.S.) and in part with federal funds from the NCI/NIH under Contract No. HHSN261200800001E. This publication was made possible with help from the Harvard University Center for AIDS Research (CFAR), an NIH funded program (P30-AI060354), which is supported by the following NIH co-funding and participating institutes and centers: NIAID, NCI, NICHD, NHLBI, NIDA, NIMH, NIA, NIDDK, NIGMS, FIC, and OAR. P.B. is a Jenner Institute Investigator.

## FIGURE LEGENDS

**Figure 1. Heat map illustrating normalized data from the validation study.** PBMC from naïve rhesus macaques (n=11) and naïve AGM (n=6) were cultured in RPMI only or stimulated with CLO-97, LPS, PHA, or PMA/Ionomycin. The median fold-change in levels of each analyte was calculated as stimulated over unstimulated and normalized to the highest response across both species and stimulation conditions. The spectrum of median fold-change is represented by the red as the maximum response and blue as the minimum response across both types of NHP for each analyte.

**Figure 2. Dynamics of SIV viremia and correlation of peak and setpoint viremia.** RM were infected with SIVmac251 (n=10) or with SIVmac239 $\Delta$ *nef* (n=10); AGM were infected with SIVsab9315BR (n=6). **(A)** Magnitude of SIV viremia in the first three months post challenge. Individual animals are depicted by broken lines; the solid lines represent the median values. **(B)** Pearson correlation of area under the curve (AUC) viremia during the first 4 weeks following SIV infection versus setpoint viremia at three months after infection.

**Figure 3. Graphic representation of the analytes up-regulated in the different SIV infection models.** Positive cytokine and chemokine responses were defined in each animal as levels >95% confidence interval above the baseline mean for each species tested, 2 $\times$  the baseline value for each individual animal, and >5 pg/ml. If more than 50% animals exhibited a positive response for a given analyte, up-regulation of that analyte was determined to be positive for the group. The positive responses observed in each SIV

model are denoted in the circles for AGM sab9135BR (red), RM SIVmac251 (blue) and RM SIVmac239 $\Delta$ nef (green). The common responses are represented in the intersection of the SIV model circles. The cytokines that were not found to be up-regulated in any of the infection models are outside of all three circles.

**Figure 4. Magnitude of analyte elevations over time after SIV infection.** Group median plasma concentrations of 15 of the 25 analytes measured are shown over time; RM SIVmac251 (blue), RM SIVmac239 $\Delta$ nef (green), AGM SIVsab9315BR (red). Analytes that did not rise above 5 pg/mL and twice the median baseline at any time point in any group are not shown. Serum amyloid A levels were measured during the first 5 weeks of infection in RM infected with SIVmac251 (n=10), RM infected with SIVmac239 $\Delta$ nef (n=9), and AGM infected with SIVsab9315BR (n=6). One of the SIVmac239 $\Delta$ nef-infected RM was not included in the SAA evaluation due to too low sample volume.

593 **References**

- 594 1. **Weissman, D., T. D. Barker, and A. S. Fauci.** 1996. The efficiency of acute  
595 infection of CD4+ T cells is markedly enhanced in the setting of antigen-specific  
596 immune activation. *The Journal of experimental medicine* **183**:687-692.
- 597 2. **McMichael, A. J., P. Borrow, G. D. Tomaras, N. Goonetilleke, and B. F.**  
598 **Haynes.** 2010. The immune response during acute HIV-1 infection: clues for  
599 vaccine development. *Nature reviews. Immunology* **10**:11-23.
- 600 3. **Fiebig, E. W., D. J. Wright, B. D. Rawal, P. E. Garrett, R. T. Schumacher, L.**  
601 **Peddada, C. Heldebrant, R. Smith, A. Conrad, S. H. Kleinman, and M. P.**  
602 **Busch.** 2003. Dynamics of HIV viremia and antibody seroconversion in plasma  
603 donors: implications for diagnosis and staging of primary HIV infection. *Aids*  
604 **17**:1871-1879.
- 605 4. **Stacey, A. R., P. J. Norris, L. Qin, E. A. Haygreen, E. Taylor, J. Heitman, M.**  
606 **Lebedeva, A. DeCamp, D. Li, D. Grove, S. G. Self, and P. Borrow.** 2009.  
607 Induction of a striking systemic cytokine cascade prior to peak viremia in acute  
608 human immunodeficiency virus type 1 infection, in contrast to more modest and  
609 delayed responses in acute hepatitis B and C virus infections. *Journal of virology*  
610 **83**:3719-3733.
- 611 5. **Kramer, H. B., K. J. Lavender, L. Qin, A. R. Stacey, M. K. Liu, K. di Gleria, A.**  
612 **Simmons, N. Gasper-Smith, B. F. Haynes, A. J. McMichael, P. Borrow, and**  
613 **B. M. Kessler.** 2010. Elevation of intact and proteolytic fragments of acute phase  
614 proteins constitutes the earliest systemic antiviral response in HIV-1 infection.  
615 *PLoS pathogens* **6**:e1000893.
- 616 6. **Katsikis, P. D., Y. M. Mueller, and F. Villinger.** 2011. The cytokine network of  
617 acute HIV infection: a promising target for vaccines and therapy to reduce viral  
618 set-point? *PLoS pathogens* **7**:e1002055.
- 619 7. **Desrosiers, R. C., and N. L. Letvin.** 1987. Animal models for acquired  
620 immunodeficiency syndrome. *Reviews of infectious diseases* **9**:438-446.
- 621 8. **Sodora, D. L., J. S. Allan, C. Apetrei, J. M. Brenchley, D. C. Douek, J. G.**  
622 **Else, J. D. Estes, B. H. Hahn, V. M. Hirsch, A. Kaur, F. Kirchhoff, M. Muller-**  
623 **Trutwin, I. Pandrea, J. E. Schmitz, and G. Silvestri.** 2009. Toward an AIDS  
624 vaccine: lessons from natural simian immunodeficiency virus infections of African  
625 nonhuman primate hosts. *Nature medicine* **15**:861-865.
- 626 9. **Lifson, J. D., M. A. Nowak, S. Goldstein, J. L. Rossio, A. Kinter, G. Vasquez,**  
627 **T. A. Wiltout, C. Brown, D. Schneider, L. Wahl, A. L. Lloyd, J. Williams, W.**  
628 **R. Elkins, A. S. Fauci, and V. M. Hirsch.** 1997. The extent of early viral  
629 replication is a critical determinant of the natural history of simian  
630 immunodeficiency virus infection. *Journal of virology* **71**:9508-9514.
- 631 10. **Daniel, M. D., F. Kirchhoff, S. C. Czajak, P. K. Sehgal, and R. C. Desrosiers.**  
632 1992. Protective effects of a live attenuated SIV vaccine with a deletion in the nef  
633 gene. *Science* **258**:1938-1941.
- 634 11. **Mohri, H., S. Bonhoeffer, S. Monard, A. S. Perelson, and D. D. Ho.** 1998.  
635 Rapid turnover of T lymphocytes in SIV-infected rhesus macaques. *Science*  
636 **279**:1223-1227.

12. **Rosenzweig, M., M. A. DeMaria, D. M. Harper, S. Friedrich, R. K. Jain, and R. P. Johnson.** 1998. Increased rates of CD4(+) and CD8(+) T lymphocyte turnover in simian immunodeficiency virus-infected macaques. *Proceedings of the National Academy of Sciences of the United States of America* **95**:6388-6393.
13. **Picker, L. J., S. I. Hagen, R. Lum, E. F. Reed-Inderbitzin, L. M. Daly, A. W. Sylwester, J. M. Walker, D. C. Siess, M. Piatak, Jr., C. Wang, D. B. Allison, V. C. Maino, J. D. Lifson, T. Kodama, and M. K. Axthelm.** 2004. Insufficient production and tissue delivery of CD4+ memory T cells in rapidly progressive simian immunodeficiency virus infection. *The Journal of experimental medicine* **200**:1299-1314.
14. **Okoye, A., H. Park, M. Rohankhedkar, L. Coyne-Johnson, R. Lum, J. M. Walker, S. L. Planer, A. W. Legasse, A. W. Sylwester, M. Piatak, Jr., J. D. Lifson, D. L. Sodora, F. Villinger, M. K. Axthelm, J. E. Schmitz, and L. J. Picker.** 2009. Profound CD4+/CCR5+ T cell expansion is induced by CD8+ lymphocyte depletion but does not account for accelerated SIV pathogenesis. *The Journal of experimental medicine* **206**:1575-1588.
15. **Pandrea, I., and C. Apetrei.** 2010. Where the wild things are: pathogenesis of SIV infection in African nonhuman primate hosts. *Current HIV/AIDS reports* **7**:28-36.
16. **Lederer, S., D. Favre, K. A. Walters, S. Proll, B. Kanwar, Z. Kasakow, C. R. Baskin, R. Palermo, J. M. McCune, and M. G. Katze.** 2009. Transcriptional profiling in pathogenic and non-pathogenic SIV infections reveals significant distinctions in kinetics and tissue compartmentalization. *PLoS pathogens* **5**:e1000296.
17. **Cumont, M. C., O. Diop, B. Vaslin, C. Elbim, L. Viollet, V. Monceaux, S. Lay, G. Silvestri, R. Le Grand, M. Muller-Trutwin, B. Hurtrel, and J. Estaquier.** 2008. Early divergence in lymphoid tissue apoptosis between pathogenic and nonpathogenic simian immunodeficiency virus infections of nonhuman primates. *Journal of virology* **82**:1175-1184.
18. **Bosinger, S. E., K. A. Hosiawa, M. J. Cameron, D. Persad, L. Ran, L. Xu, M. R. Boulassel, M. Parenteau, J. Fournier, E. W. Rud, and D. J. Kelvin.** 2004. Gene expression profiling of host response in models of acute HIV infection. *Journal of immunology* **173**:6858-6863.
19. **Kornfeld, C., M. J. Ploquin, I. Pandrea, A. Faye, R. Onanga, C. Apetrei, V. Poaty-Mavoungou, P. Rouquet, J. Estaquier, L. Mortara, J. F. Desoutter, C. Butor, R. Le Grand, P. Roques, F. Simon, F. Barre-Sinoussi, O. M. Diop, and M. C. Muller-Trutwin.** 2005. Antiinflammatory profiles during primary SIV infection in African green monkeys are associated with protection against AIDS. *The Journal of clinical investigation* **115**:1082-1091.
20. **Abel, K., D. M. Rocke, B. Chohan, L. Fritts, and C. J. Miller.** 2005. Temporal and anatomic relationship between virus replication and cytokine gene expression after vaginal simian immunodeficiency virus infection. *Journal of virology* **79**:12164-12172.
21. **Gupta, M., A. MacNeil, Z. D. Reed, P. E. Rollin, and C. F. Spiropoulou.** 2012. Serology and cytokine profiles in patients infected with the newly discovered Bundibugyo ebolavirus. *Virology* **423**:119-124.

22. **Almansa, R., M. Sanchez-Garcia, A. Herrero, S. Calzada, V. Roig, J. Barbado, L. Rico, F. Bobillo, J. M. Eiros, V. Iglesias, R. O. de Lejarazu, and J. F. Bermejo-Martin.** 2011. Host response cytokine signatures in viral and nonviral acute exacerbations of chronic obstructive pulmonary disease. *Journal of interferon & cytokine research : the official journal of the International Society for Interferon and Cytokine Research* **31**:409-413.
23. **Broussard, S. R., S. I. Staprans, R. White, E. M. Whitehead, M. B. Feinberg, and J. S. Allan.** 2001. Simian immunodeficiency virus replicates to high levels in naturally infected African green monkeys without inducing immunologic or neurologic disease. *Journal of virology* **75**:2262-2275.
24. **Zahn, R. C., M. D. Rett, M. Li, H. Tang, B. Koriath-Schmitz, H. Balachandran, R. White, S. Pryputniewicz, N. L. Letvin, A. Kaur, D. C. Montefiori, A. Carville, V. M. Hirsch, J. S. Allan, and J. E. Schmitz.** 2010. Suppression of adaptive immune responses during primary SIV infection of sabaues African green monkeys delays partial containment of viremia but does not induce disease. *Blood* **115**:3070-3078.
25. 2011. *Guide for the Care and Use of Laboratory Animals*, 8th ed, Washington (DC).
26. **Cline, A. N., J. W. Bess, M. Piatak, Jr., and J. D. Lifson.** 2005. Highly sensitive SIV plasma viral load assay: practical considerations, realistic performance expectations, and application to reverse engineering of vaccines for AIDS. *Journal of medical primatology* **34**:303-312.
27. **Benjamini, Y.** 2010. Discovering the false discovery rate. *Journal of the Royal Statistical Society, Series B* **72**:405-416.
28. **Staprans, S. I., P. J. Dailey, A. Rosenthal, C. Horton, R. M. Grant, N. Lerche, and M. B. Feinberg.** 1999. Simian immunodeficiency virus disease course is predicted by the extent of virus replication during primary infection. *Journal of virology* **73**:4829-4839.
29. **Ling, Z. D., E. Yeoh, B. T. Webb, K. Farrell, J. Doucette, and D. S. Matheson.** 1993. Intravenous immunoglobulin induces interferon-gamma and interleukin-6 in vivo. *Journal of clinical immunology* **13**:302-309.
30. **Aukrust, P., K. Hestdal, E. Lien, V. Bjerkeli, I. Nordoy, T. Espevik, F. Muller, and S. S. Froland.** 1997. Effects of intravenous immunoglobulin in vivo on abnormally increased tumor necrosis factor-alpha activity in human immunodeficiency virus type 1 infection. *The Journal of infectious diseases* **176**:913-923.
31. **Aukrust, P., S. S. Froland, N. B. Liabakk, F. Muller, I. Nordoy, C. Haug, and T. Espevik.** 1994. Release of cytokines, soluble cytokine receptors, and interleukin-1 receptor antagonist after intravenous immunoglobulin administration in vivo. *Blood* **84**:2136-2143.
32. **Roberts, L., J. A. Passmore, C. Williamson, F. Little, L. M. Bebell, K. Mlisana, W. A. Burgers, F. van Loggerenberg, G. Walzl, J. F. Djoba Siawaya, Q. A. Karim, and S. S. Karim.** 2010. Plasma cytokine levels during acute HIV-1 infection predict HIV disease progression. *Aids* **24**:819-831.
33. **Mueller, Y. M., D. H. Do, S. R. Altork, C. M. Artlett, E. J. Gracely, C. D. Katsetos, A. Legido, F. Villinger, J. D. Altman, C. R. Brown, M. G. Lewis, and**

- P. D. Katsikis.** 2008. IL-15 treatment during acute simian immunodeficiency virus (SIV) infection increases viral set point and accelerates disease progression despite the induction of stronger SIV-specific CD8+ T cell responses. *Journal of immunology* **180**:350-360.
34. **Manetti, R., F. Gerosa, M. G. Giudizi, R. Biagiotti, P. Parronchi, M. P. Piccinni, S. Sampognaro, E. Maggi, S. Romagnani, and G. Trinchieri.** 1994. Interleukin 12 induces stable priming for interferon gamma (IFN-gamma) production during differentiation of human T helper (Th) cells and transient IFN-gamma production in established Th2 cell clones. *The Journal of experimental medicine* **179**:1273-1283.
  35. **Manetti, R., P. Parronchi, M. G. Giudizi, M. P. Piccinni, E. Maggi, G. Trinchieri, and S. Romagnani.** 1993. Natural killer cell stimulatory factor (interleukin 12 [IL-12]) induces T helper type 1 (Th1)-specific immune responses and inhibits the development of IL-4-producing Th cells. *The Journal of experimental medicine* **177**:1199-1204.
  36. **Boyer, J. D., P. C. Maciag, R. Parkinson, L. Wu, M. G. Lewis, D. B. Weiner, and Y. Paterson.** 2006. Rhesus macaques with high levels of vaccine induced IFN-gamma producing cells better control viral set-point following challenge with SIV239. *Vaccine* **24**:4498-4502.
  37. **Clements, J. E., J. L. Mankowski, L. Gama, and M. C. Zink.** 2008. The accelerated simian immunodeficiency virus macaque model of human immunodeficiency virus-associated neurological disease: from mechanism to treatment. *Journal of neurovirology* **14**:309-317.
  38. **Breen, E. C., A. R. Rezai, K. Nakajima, G. N. Beall, R. T. Mitsuyasu, T. Hirano, T. Kishimoto, and O. Martinez-Maza.** 1990. Infection with HIV is associated with elevated IL-6 levels and production. *Journal of immunology* **144**:480-484.
  39. **Ramesh, G., X. Alvarez, J. T. Borda, P. P. Aye, A. A. Lackner, and K. Sestak.** 2005. Visualizing cytokine-secreting cells in situ in the rhesus macaque model of chronic gut inflammation. *Clinical and diagnostic laboratory immunology* **12**:192-197.
  40. **French, M. A., A. Cozzi-Lepri, R. C. Arduino, M. Johnson, A. C. Achhra, A. Landay, and I. S. S. Group.** 2015. Plasma levels of cytokines and chemokines and the risk of mortality in HIV-infected individuals: a case-control analysis nested in a large clinical trial. *Aids* **29**:847-851.
  41. **McDonald, B., S. Moyo, L. Gabaitiri, S. Gaseitsiwe, H. Bussmann, J. R. Koethe, R. Musonda, J. Makhema, V. Novitsky, R. G. Marlink, C. W. Wester, and M. Essex.** 2013. Persistently elevated serum interleukin-6 predicts mortality among adults receiving combination antiretroviral therapy in Botswana: results from a clinical trial. *AIDS research and human retroviruses* **29**:993-999.
  42. **Tao, B., and P. N. Fultz.** 1999. Pathogenicity and comparative evolution in vivo of the transitional quasispecies SIVsmmPBj8. *Virology* **259**:166-175.
  43. **Rosser, E. C., K. Oleinika, S. Tonon, R. Doyle, A. Bosma, N. A. Carter, K. A. Harris, S. A. Jones, N. Klein, and C. Mauri.** 2014. Regulatory B cells are induced by gut microbiota-driven interleukin-1beta and interleukin-6 production. *Nature medicine* **20**:1334-1339.

44. **Gordon, S. N., N. R. Klatt, S. E. Bosinger, J. M. Brenchley, J. M. Milush, J. C. Engram, R. M. Dunham, M. Paiardini, S. Klucking, A. Danesh, E. A. Strobert, C. Apetrei, I. V. Pandrea, D. Kelvin, D. C. Douek, S. I. Staprans, D. L. Sodora, and G. Silvestri.** 2007. Severe depletion of mucosal CD4+ T cells in AIDS-free simian immunodeficiency virus-infected sooty mangabeys. *Journal of immunology* **179**:3026-3034.
45. **Hao, X. P., C. M. Lucero, B. Turkbey, M. L. Bernardo, D. R. Morcock, C. Deleage, C. M. Trubey, J. Smedley, N. R. Klatt, L. D. Giavedoni, J. Kristoff, A. Xu, G. Q. Del Prete, B. F. Keele, S. S. Rao, W. G. Alvord, P. L. Choyke, J. D. Lifson, J. M. Brenchley, C. Apetrei, I. Pandrea, and J. D. Estes.** 2015. Experimental colitis in SIV-uninfected rhesus macaques recapitulates important features of pathogenic SIV infection. *Nature communications* **6**:8020.
46. **Swan, Z. D., E. R. Wonderlich, and S. M. Barratt-Boyes.** 2016. Macrophage accumulation in gut mucosa differentiates AIDS from chronic SIV infection in rhesus macaques. *European journal of immunology* **46**:446-454.
47. **Micci, L., E. S. Ryan, R. Fromentin, S. E. Bosinger, J. L. Harper, T. He, S. Paganini, K. A. Easley, A. Chahroudi, C. Benne, S. Gumber, C. S. McGary, K. A. Rogers, C. Deleage, C. Lucero, S. N. Byraredy, C. Apetrei, J. D. Estes, J. D. Lifson, M. Piatak, Jr., N. Chomont, F. Villinger, G. Silvestri, J. M. Brenchley, and M. Paiardini.** 2015. Interleukin-21 combined with ART reduces inflammation and viral reservoir in SIV-infected macaques. *The Journal of clinical investigation* **125**:4497-4513.



Figure 1

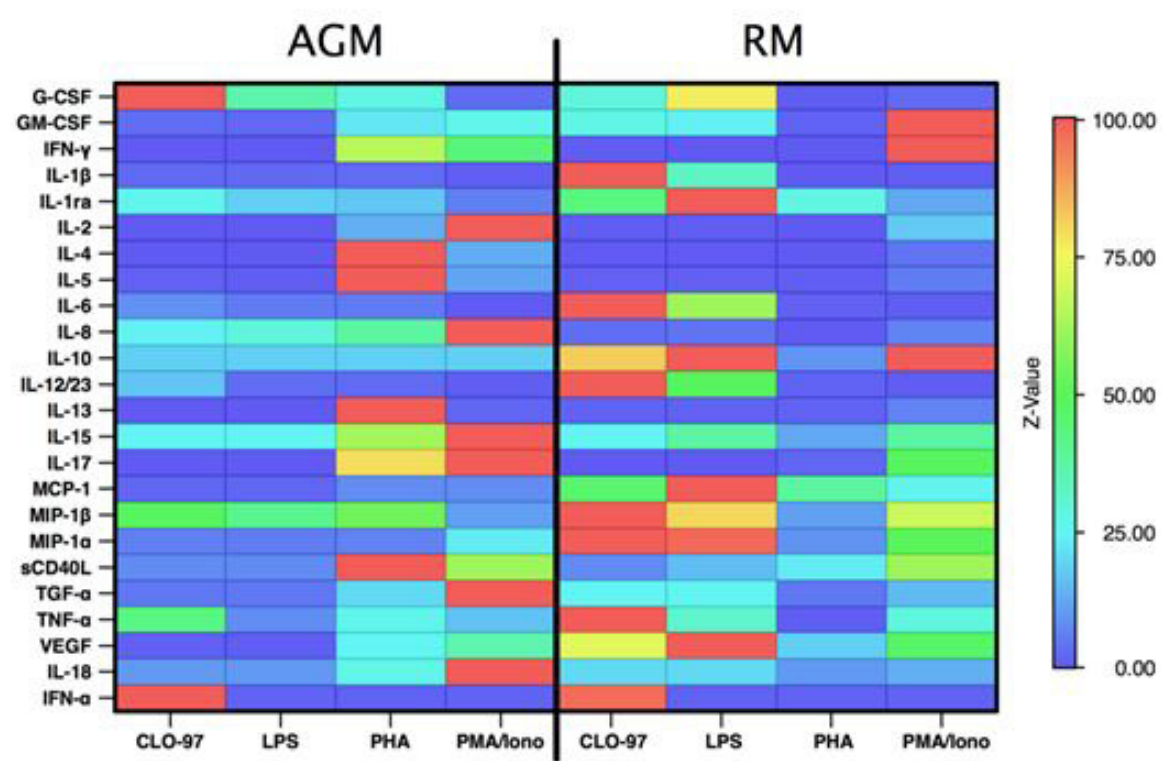
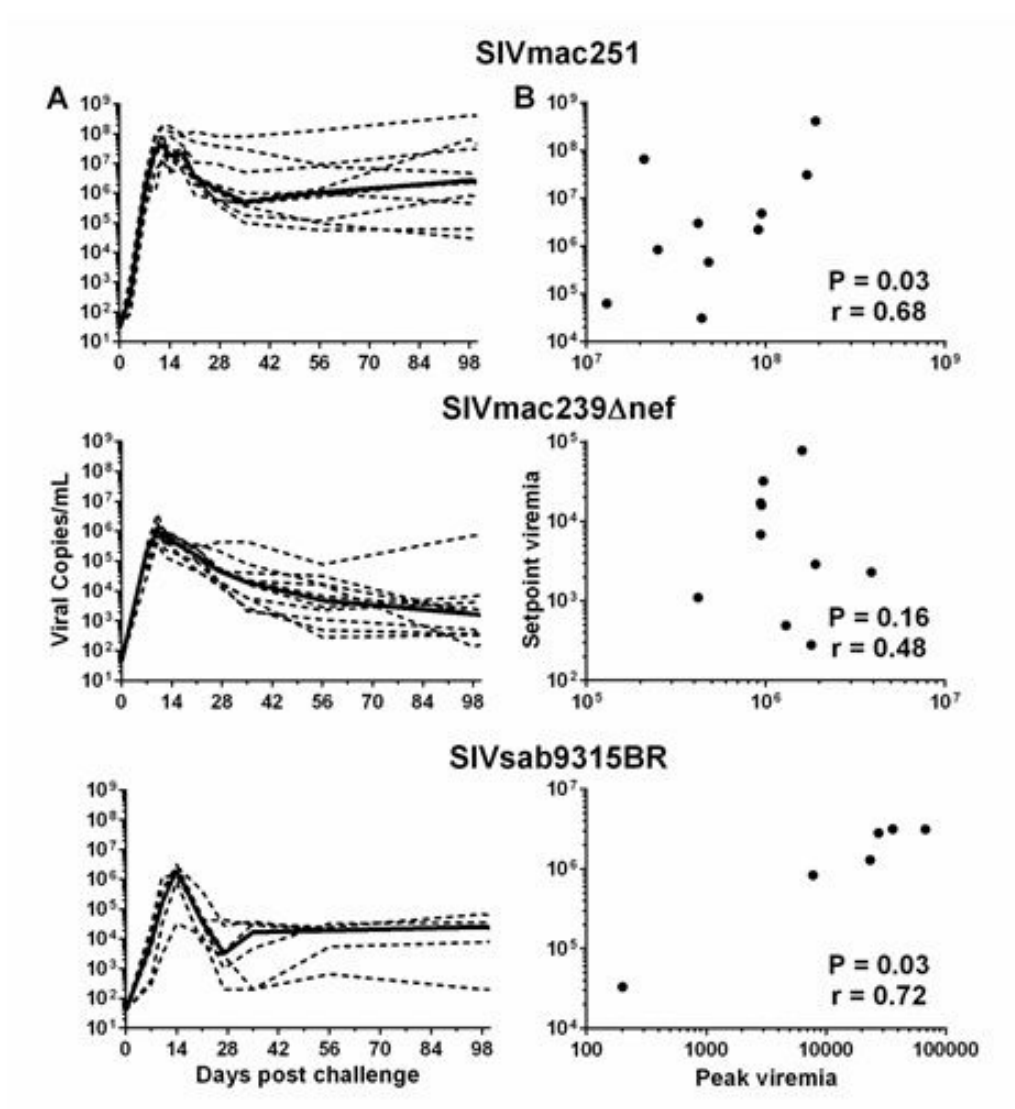
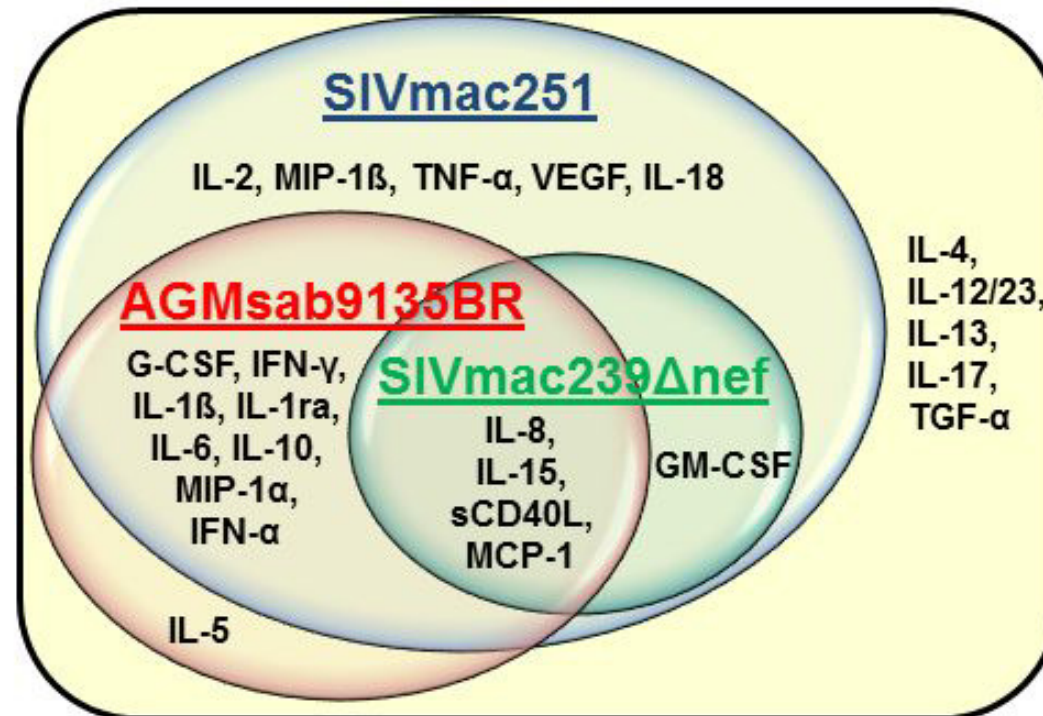
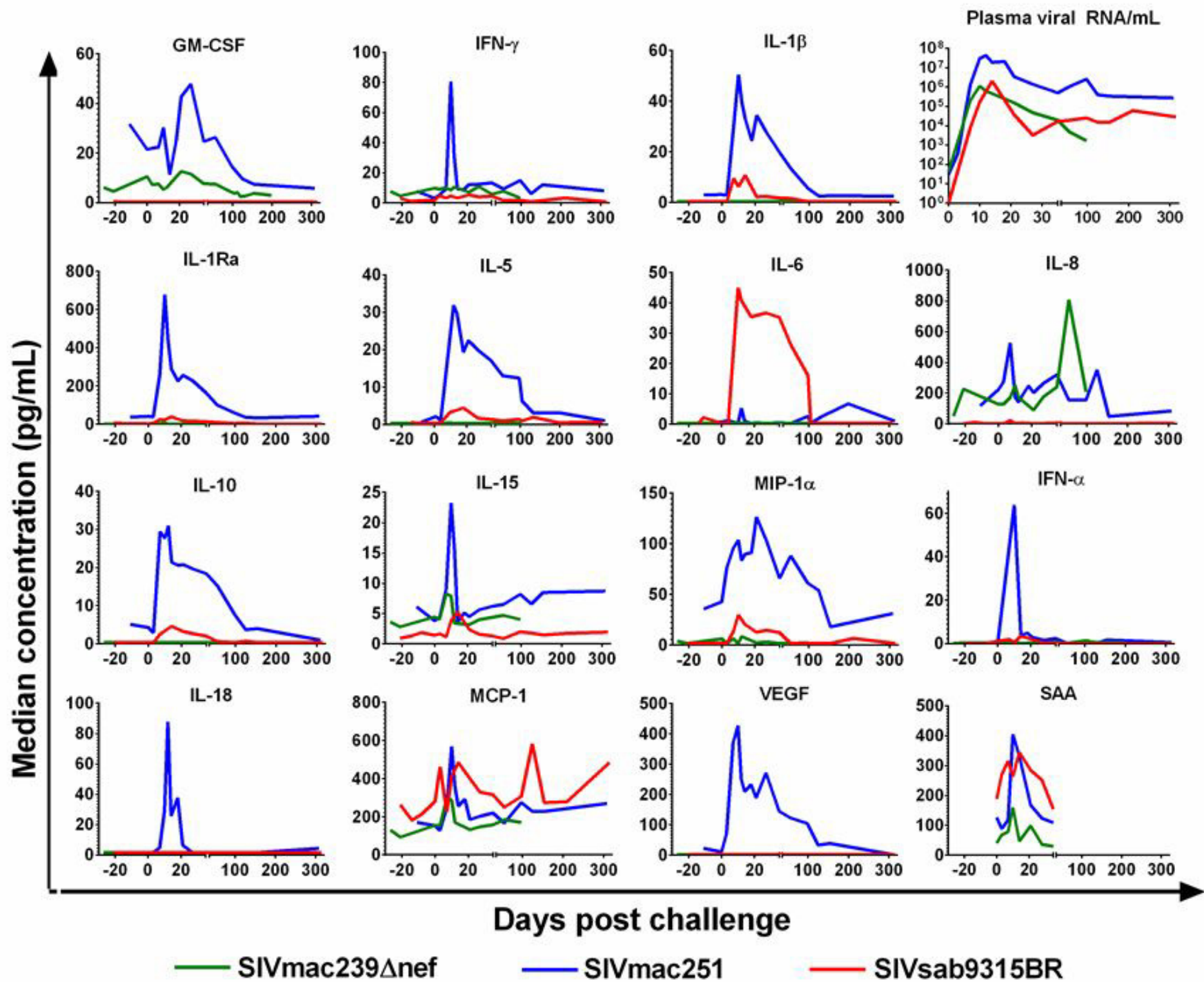


Figure 2







**Table 1. Multiplex kit cross-species reactivity.**

Median fold-change	Human <sup>a</sup>				AGM <sup>a</sup>				RM <sup>a</sup>			
	CLO-97	LPS	PHA	PMA/Iono	CLO-97	LPS	PHA	PMA/Iono <sup>b</sup>	CLO-97	LPS	PHA	PMA/Iono
G-CSF	<b>2961<sup>c</sup></b>	<b>5363</b>	26	15	<b>162</b>	<b>58</b>	<b>44</b>	5	<b>46</b>	<b>124</b>	1	5
GM-CSF	<b>155</b>	<b>182</b>	77	<b>2673</b>	14	9	<b>90</b>	<b>108</b>	<b>112</b>	<b>98</b>	6	<b>410</b>
IFN- $\gamma$	<b>100</b>	34	<b>38</b>	<b>4993</b>	7	11	<b>3110</b>	<b>2115</b>	<b>23</b>	12	<b>14</b>	<b>4776</b>
IL-1 $\beta$	<b>8856</b>	<b>2910</b>	10	260	<b>73</b>	<b>84</b>	<b>97</b>	14	<b>3637</b>	<b>1172</b>	7	18
IL-1ra	<b>106</b>	<b>123</b>	13	31	<b>17</b>	12	<b>11</b>	4	<b>28</b>	<b>65</b>	<b>18</b>	8
IL-2	7	7	<b>9</b>	<b>444</b>	11	15	<b>1116</b>	<b>8514</b>	<b>31</b>	<b>40</b>	21	<b>1481</b>
IL-4	13	23	<b>225</b>	<b>769</b>	1	2	<b>2266</b>	<b>286</b>	1	1	7	<b>107</b>
IL-5	6	7	<b>103</b>	<b>596</b>	2	1	<b>198</b>	24	2	2	1	<b>11</b>
IL-6	<b>7610</b>	<b>7751</b>	1124	188	<b>253</b>	<b>157</b>	<b>160</b>	4	<b>3015</b>	<b>1861</b>	28	19
IL-8	<b>106</b>	<b>106</b>	<b>86</b>	<b>106</b>	756	925	<b>1205</b>	3199	<b>104</b>	<b>146</b>	9	<b>204</b>
IL-10	<b>180</b>	<b>607</b>	<b>48</b>	26	2	2	2	2	<b>9</b>	<b>11</b>	1	<b>11</b>
IL-12/23	<b>1701</b>	<b>855</b>	9	16	<b>297</b>	<b>57</b>	42	8	<b>1783</b>	<b>867</b>	27	13
IL-13	22	26	<b>79</b>	<b>288</b>	1	1	<b>639</b>	12	<b>9</b>	<b>9</b>	<b>9</b>	<b>42</b>
IL-15	<b>5</b>	<b>7</b>	3	<b>6</b>	2	2	5	8	2	3	1	3
IL-17	0	1	<b>12</b>	<b>20</b>	1	1	<b>497</b>	627	0	0	11	<b>305</b>
MCP-1	<b>12</b>	<b>12</b>	<b>12</b>	<b>12</b>	1	1	5	5	<b>29</b>	<b>63</b>	<b>24</b>	<b>16</b>
MIP-1 $\beta$	<b>390</b>	<b>535</b>	137	192	<b>185</b>	<b>157</b>	<b>206</b>	41	<b>382</b>	<b>305</b>	41	<b>262</b>
MIP-1 $\alpha$	<b>3693</b>	<b>4592</b>	262	<b>4561</b>	281	276	<b>300</b>	1104	<b>4770</b>	<b>4666</b>	415	<b>2410</b>
sCD40L	2	3	<b>6</b>	<b>6</b>	1	1	<b>13</b>	8	1	2	<b>3</b>	<b>8</b>
TGF- $\alpha$	<b>28</b>	<b>43</b>	<b>14</b>	2	1	1	4	20	<b>5</b>	<b>5</b>	1	3
TNF- $\alpha$	<b>7770</b>	<b>2265</b>	290	<b>3167</b>	<b>1281</b>	241	<b>787</b>	488	<b>3006</b>	<b>925</b>	18	<b>846</b>
VEGF	<b>20</b>	<b>23</b>	4	6	5	2	<b>114</b>	<b>164</b>	<b>335</b>	<b>464</b>	84	<b>219</b>
IL-18	<b>8</b>	<b>3</b>	2	3	3	3	<b>8</b>	<b>30</b>	<b>6</b>	<b>6</b>	3	<b>4</b>
IFN- $\alpha$ <sup>d</sup>	51	1	1	1	<b>77</b>	1	1	1	75	1	1	1

<sup>a</sup>Human (n=8), AGM (n=6) and RM (n=11) PBMC were cultured in RPMI only or were stimulated with CLO-97, LPS, PHA, or PMA/Ionomycin, and the fold-change in analyte level was calculated as stimulated over unstimulated.

<sup>b</sup>AGM (n=3).

<sup>c</sup>The bold values represent significant increases ( $P<0.05$ ) over baseline unstimulated (Kruskal Wallis test with Dunn's multiple comparison tests).

<sup>d</sup> Human (n=2), AGM (n=3) and RM (n=2).

**Table 2. Percentage of animals with cytokine responses after SIV infection.**

	Baseline		% responder		
	RM Upper 95% CI <sup>a</sup> (n=74)	AGM Upper 95% CI <sup>a</sup> (n=19)	RM SIVmac251 <sup>b</sup> (n=10)	RM SIV mac239 $\Delta$ nef <sup>b</sup> (n=10)	AGM SIVsab9315BR <sup>b</sup> (n=6)
<b>G-CSF</b>	1.3	2.6	<b>60</b>	30	<b>50</b>
<b>GM-CSF</b>	17.0	6.5	<b>80</b>	<b>50</b>	17
<b>IFN-<math>\gamma</math></b>	11.0	5.2	<b>100</b>	30	<b>83</b>
<b>IL-1<math>\beta</math></b>	12.0	2.1	<b>100</b>	0	<b>50</b>
<b>IL-1ra</b>	40.0	22.0	<b>100</b>	0	<b>50</b>
<b>IL-2</b>	20.0	49.0	<b>50</b>	40	33
<b>IL-4</b>	0.7	1.6	0	10	17
<b>IL-5</b>	1.7	3.3	10	30	<b>50</b>
<b>IL-6</b>	1.9	18.0	<b>60</b>	10	<b>50</b>
<b>IL-8</b>	164.0	20.0	<b>60</b>	<b>90</b>	<b>50</b>
<b>IL-10</b>	2.5	2.6	<b>100</b>	0	<b>50</b>
<b>IL-12/23</b>	152.0	62.0	30	0	0
<b>IL-13</b>	0.6	1.2	30	0	17
<b>IL-15</b>	4.3	2.2	<b>100</b>	<b>50</b>	<b>67</b>
<b>IL-17</b>	0.9	0.9	40	0	17
<b>MCP-1</b>	153.0	408.0	<b>100</b>	<b>50</b>	<b>67</b>
<b>MIP-1<math>\beta</math></b>	2.5	8.5	<b>50</b>	0	0
<b>MIP-1<math>\alpha</math></b>	21.0	18.0	<b>70</b>	20	<b>67</b>
<b>sCD40L</b>	318.0	1395.0	<b>60</b>	<b>80</b>	<b>83</b>
<b>TGF-<math>\alpha</math></b>	59.0	4.0	0	0	0
<b>TNF-<math>\alpha</math></b>	19.0	2.3	<b>50</b>	0	33
<b>VEGF</b>	43.0	60.0	<b>100</b>	0	33
<b>IL-18</b>	38.0	1.8	<b>90</b>	0	17
<b>IFN-<math>\alpha</math></b>	1.7	4.1	<b>100</b>	0	<b>50</b>
<b>SAA<sup>c</sup></b>	146.7	256.5	<b>90</b>	44	<b>67</b>

<sup>a</sup>The upper 95% confidence interval was calculated by testing the baseline analyte levels in 74 untreated RM and 19 untreated AGM.

<sup>b</sup>RM infected with SIVmac251 (n=10), RM infected with SIVmac239 $\Delta$ nef (n=10), and AGM infected with SIVsab9315BR (n=6) were analyzed. The percentage of animals that at any time point had analyte levels above the upper 95% confidence of the group baseline, two-fold change over the individual baseline and were >5pg/ml is shown. Bold numbers denote groups and cytokines with more than a 50% response and therefore considered a positive response for the group.

<sup>c</sup>For the SAA evaluation, 19 RM and 6 AGM were included in the baseline analysis. One of the SIVmac239 $\Delta$ nef-infected RM was not included in the SAA evaluation due to insufficient sample volume.

**Table 3. Cytokine responses after pathogenic and non-pathogenic SIV infection in RM and AGM.**

	Rhesus macaque						African green monkey			
	Baseline Median <sup>a</sup>	IQR <sup>a</sup>	SIVmac251 Median Peak Response <sup>b</sup>	Day <sup>b</sup>	SIVmac239Δnef Median Peak Response <sup>b</sup>	Day <sup>b</sup>	Baseline Median <sup>a</sup>	IQR <sup>a</sup>	SIVsab9315BR Median Peak Response <sup>b</sup>	Day <sup>b</sup>
G-CSF	0.3	0.3 - 0.7	1.8	12	2.3	21	0.3	0.3 - 2	4.5	10
<b>GM-CSF<sup>c</sup></b>	3.4	0.3 - 15	47.8	27	12.6	21	0.6	0.3 - 7	0.5	NR <sup>d</sup>
<b>IFN-γ</b>	6.2	3 - 11	80.5	10	10.8	27	2	1 - 5	5.2	21
<b>IL-1β</b>	0.3	0.3 - 3.5	50.5	10	0.3	NR	0.3	0.3 - 3	10.8	14
<b>IL-1ra</b>	8.0	0.3 - 37	678.5	10	7.2	NR	4	2 - 18	38.4	14
IL-2	14.7	4.3 - 23	25.9	154	23.3	56	24	4 - 59	23.6	21
<b>IL-5</b>	0.3	0.1 - 0.3	31.9	10	0.5	7	0.6	0.3 - 3	4.4	14
<b>IL-6</b>	0.3	0.3 - 0.3	6.8	154	0.3	NR	0.3	0.3 - 9	45.0	7
<b>IL-8</b>	98.2	61 - 189	526.4	7	807.5	56	2	0.3 - 9	22.4	7
<b>IL-10</b>	0.3	0.2 - 0.3	31.0	12	0.3	NR	0.3	0.3 - 0.6	4.6	14
<b>IL-15</b>	4.0	2.4 - 5.5	23.2	10	8.3	7	0.8	0.3 - 2	5.2	14
<b>MCP-1</b>	130.4	102 - 168	568.4	10	291.9	7	316	188 - 378	582.6	125
<b>MIP-1α</b>	7.0	0.3 - 19	126.4	21	8.5	NR	2	2 - 13	29.8	10
MIP-1β	0.3	0.3 - 0.8	6.4	154	0.3	ND <sup>e</sup>	0.3	0.3 - 0.3	0.3	ND
sCD40L	209.1	119 - 393	269.7	NR	348.5	56	392	149 - 1047	551.5	21
TNF-α	3.4	0.3 - 13	7.0	NR	0.9	NR	0.3	0.3 - 1	1.8	NR
VEGF	0.3	0.3 - 7.5	427.3	10	1.6	NR	2	2 - 56	1.6	NR
IL-18	0.3	0.3 - 9.6	87.8	12	1.6	NR	2	2 - 2	1.6	NR
<b>IFN-α</b>	1.0	0.6 - 2.1	63.8	10	1.9	NR	1	1 - 2	3.4	12
<b>SAA</b>	40	20 - 87	452	7	80	7	39	19 - 65.5	316.6	7

<sup>a</sup>Median and lower and upper quartiles for the baseline responses. Values are reported in pg/mL.

<sup>b</sup>The peak median response for each group, which differed between animals, analytes, and groups, is shown with its corresponding day after infection.

<sup>c</sup>Analytes with significant differences (determined by ANOVA followed by Tukey Honestly Significant Differences (HSD)) in peak levels of cytokine between the three different infection models are shown in bold. (IL-4, IL-12, IL-13, IL-17, and TGF-α were not found to be up-regulated in >50% animals and are not included in this table).

<sup>d</sup>NR: Analytes that did not reach the upper 95% CI of the group baseline did not exhibit a two-fold change over the individual baseline at any time point and did not exceed 5 pg/ml, the criteria for response up-regulation.

<sup>e</sup>ND: Analytes had no detectable response.

**Table 4. Cytokine responses during early infection that correlate with viral setpoint.**

	<b>R<sup>2</sup></b>	<b>P-value</b>	<b>FDR</b>
<b>SIVsab9315BR</b>			
IL-6	0.85 <sup>a</sup>	0.009	0.07
MCP-1	0.85	0.009	0.07
<i>sCD40L</i>	<i>0.64</i>	<i>0.057</i>	<i>0.24</i>
<i>IL-1β</i>	<i>0.61</i>	<i>0.068</i>	<i>0.24</i>
<i>IL-8</i>	<i>0.54</i>	<i>0.095</i>	<i>0.27</i>
<b>SIVmac239Δnef</b>			
MCP-1	0.75	0.001	0.003
sCD40L	0.76	0.001	0.003
<i>IL-8</i>	<i>0.221</i>	<i>0.17</i>	<i>0.28</i>
<b>SIVmac251</b>			
IFN-γ	0.83	0.0003	0.004
IL-18	0.81	0.0004	0.004
G-CSF	0.64	0.006	0.04
IL-15	0.59	0.009	0.05
MIP-1β	0.55	0.015	0.06
SAA	0.421	0.04	0.11
MCP-1	0.405	0.048	0.11
<i>VEGF</i>	<i>0.41</i>	<i>0.05</i>	<i>0.11</i>
<i>IL-10</i>	<i>0.287</i>	<i>0.11</i>	<i>0.23</i>
<i>IL-6</i>	<i>0.257</i>	<i>0.13</i>	<i>0.25</i>
<i>MIP-1α</i>	<i>0.246</i>	<i>0.145</i>	<i>0.25</i>

<sup>a</sup>For each analyte, area under the curve (AUC) was calculated for the first 4 weeks of infection and a regression analysis was done comparing this value with setpoint viremia. Non-significant *P* values (*P* values between 0.05 and 0.2; possibly representing a trend) are shown below the dotted lines in italics.