Cellular & Molecular Immunology (2012) 9, 410–416

www.nature.com/cmi

RESEARCH ARTICLE

Inhibitory effects of chloroquine on the activation of plasmacytoid dendritic cells in SIVmac239-infected Chinese rhesus macaques

Jian-Ping Ma[1,2], Hou-Jun Xia[1,2], Gao-Hong Zhang[1], Jian-Bao Han[1], Li-Guo Zhang[3] and Yong-Tang Zheng[1]

It is currently widely accepted that immune activation in HIV-infected individuals leads to a severe loss of CD4$^+$ T cells and the progression to AIDS. However, the underlying mechanism of this immune activation remains unclear. Experimental data suggest that the activation of plasmacytoid dendritic cells (pDCs) by plasma viremia may play a critical role in HIV-induced immune activation. In this study, we found that the level of immune activation was higher in the late phase of SIVmac239 infection compared with chronic infection, which suggests that immune activation might be related to disease progression in SIVmac239-infected non-human primate models. Our work also showed that chloroquine could effectively inhibit the activation of pDCs *in vitro* and *in vivo*. However, chloroquine treatment of SIVmac239-infected macaques had no significant influence on the Cellular composition of peripheral blood in these animals.
Cellular & Molecular Immunology (2012) **9,** 410–416; doi:10.1038/cmi.2012.22; published online 13 August 2012

Keywords: Chinese rhesus macaque; chloroquine; immune activation; pDC; SIV

INTRODUCTION

The pathogenesis of AIDS is extremely complex, so our understanding of its underlying mechanism is very limited. Implementing a suitable animal model is crucial to the investigation of the mechanisms of AIDS disease progression. Non-human primates share many genetic and physiological similarities with humans and so are widely used as animal models for human diseases. For example, it has been reported that rhesus macaques possess Toll-like receptor (TLR) expression patterns in their antigen-presenting cells, such as dendritic cells, macrophages and B cells, that are similar to their human counterparts.[1]

Recently, it has been accepted that in addition to the direct infection of CD4$^+$ T lymphocytes by HIV virions, chronic immune activation in HIV-infected human and simian immunodeficiency virus (SIV)-infected non-natural hosts, such as rhesus macaques and cynomolgus macaques, may greatly contribute to the pathogenesis of AIDS.[2–7] Accumulated evidence suggests that large amounts of type I interferons (IFNs) produced by activated plasmacytoid dendritic cells (pDCs) might play a critical role in this chronic immune activation during HIV-1 infection. Clinical strategies aimed at inhibiting pDC activation could effectively counter disease progression in HIV patients.

Chloroquine, which is a well-known antimalarial drug discovered in the 1930s, was recently reported to have additional functions.[8] It was shown to exert direct antiviral effects by inhibiting viral replication that was dependent on the low pH level in acidic organelles, such as lysosomes, endosomes and Golgi vesicles.[8] In addition to the direct inhibition of viral replication, chloroquine has also been used to control HIV-1-induced pDC activation.[9] Studies have shown that the activation of pDCs during HIV infection relies on the endocytosis of virions into the endosome of the host cell.[10] The virions are then degraded by proteases in the endosome and the single-stranded RNA of HIV is exposed to TLR7 molecules that are expressed in the endosomes of pDCs. The TLR pathway is then activated, and IFN-α is secreted by these activated pDCs. As chloroquine could potentially inhibit the acidification of the endosome, where the most important steps in pDC activation occurs, it might have potential uses as an anti-AIDS drug by acting to inhibit immune activation and slow disease progression.

Recently, several reports have suggested the possible benefit of the use of chloroquine in the inhibition of immune activation *in vitro* and *in vivo* for the treatment of HIV infection.[9,11,12] However, the evaluation of a new function of this classic drug in SIV-infected non-human primates, which is the most relevant animal model in AIDS research, has not been reported. SIV-infected macaques serve as an important animal model that can effectively mimic AIDS disease progression because these primates share similar TLR expression patterns with humans.[1] In this study, the ability of chloroquine to inhibit pDC activation was tested by us. The results showed that this drug can effectively inhibit the activation of pDCs derived from SIVmac239-infected Chinese rhesus macaques *in vitro* and *in vivo*, while its influence on AIDS disease progression remains unclear.

[1]Key Laboratory of Animal Models and Human Disease Mechanisms of the Chinese Academy of Sciences & Yunnan Province, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, China; [2]Graduate University of the Chinese Academy of Sciences, Beijing, China and [3]CAS Key Laboratory of Infection and Immunity, Institute of Biophysics, Chinese Academy of Sciences, Beijing, China
Correspondence: Dr YT Zheng, Kunming Institute of Zoology, Chinese Academy of Sciences, 32 Jiaochang Donglu, Kunming 650223, China.
E-mail: zhengyt@mail.kiz.ac.cn
Received 12 April 2012; revised 22 May 2012; accepted 8 June 2012

MATERIALS AND METHODS

Animals

All animal and *in vitro* procedures were performed using standard protocols according to the guidelines approved by the Ethics Committee of Kunming Institute of Zoology, Chinese Academy of Sciences (CAS) and in accordance with the recommendations of the Weatherall reports: *The use of non-human primates in research*.

Three SIV-negative (06025, 06065 and 06067) and six SIVmac239-infected Chinese rhesus macaques (00067, 00079, 01035, 04029, 04039 and 05049) were used in this study. All of the Chinese rhesus macaques were male and were obtained from the Kunming Primate Research Center, Kunming Institute of Zoology, CAS. Naive animals were screened and found to be negative for simian type D retrovirus and SIV using antibody ELISA and PCR tests prior to experimental use. The SIVmac239-infected Chinese rhesus macaques were treated as previously described.[13]

The SIVmac239-infected macaques were housed at the animal biosafety level-3 laboratory. The three macaques (00067, 00079 and 01035) that were found to have $CD4^+$ T-cell counts below 500 events per microliter were considered to be in the late infection stage while the others were considered to be chronically infected. To evaluate the ability of chloroquine to inhibit immune activation in SIV-infected macaques *in vivo*, all three macaques were treated with 25 mg/kg chloroquine every other day for 30 days. Peripheral blood was got at the time points of −15, 0, 15, 30 and 60(−15 means 15 days before the chloroquine treatment while 0 means the day of drug treatment started).

Peripheral blood mononuclear cell (PBMC) isolation and stimulation assays

PBMCs were isolated by Ficoll-Paque (GE Healthcare, Piscataway, New Jersey, United States) density gradient centrifugation from whole blood samples. The PBMCs were cultured in 24-well plates (Costar, New York, United States) at 1×10^6 cells/ml in RPMI-1640 (Gibco, New York, United States) containing 10% fetal bovine serum and several TLR ligands (InvivoGen, San Diego, California, United States) including CpG2395 (5 μM), ODN2088 (50 μM) and R837 (5 μg), depending on the experiment. In the virion stimulation tests, the SIVmac239 virions were used at a p27 concentration of 200 ng/ml, while the herpes simplex virus (HSV) virions were used as previously described.[13] A chloroquine diphosphate salt (Sigma, Oakville, Ontario, Canada) solution was used in pDC activation tests at a concentration of 100 μM for 24 h and in T-cell activation tests at a concentration of 5 μM as described by Martinson *et al*.[9]

Flow cytometry

The mouse antihuman monoclonal antibodies used in our study are described in Table 1. The methods used for flow cytometry analysis of the dendritic cells and T cell subsets have been previously described.[13,14] Briefly, 50-μl whole blood samples were used in each analysis. After incubating blood samples with antibodies (Anti HLA-DR, CD123 antibodies for pDC while anti CD3, CD4 and CD8 antibodies for T cells) in the dark for 15 min, samples were treated with FACS lysing solution (BD Biosciences, San Jose, California, United States) for 10 min at room temperature. The remaining white blood cells were then washed at 500*g* for 5 min. The cell pellets were then re-suspended in Dulbecco's phosphate-buffered saline-containing bovine serum albumin (0.2%) (BD Biosciences). All the samples were analyzed using an FACS-Calibur flow cytometer (BD Biosciences) and the data were analyzed using CellQuest software (BD Biosciences).

Table 1 The monoclonal antibodies used in this study

Antigen	*Source*	*Clone*	*Isotype*
CD3-PE	Miltenyi Biotec	10D12	IgG1
CD4-PerCP	BD Biosciences	MT-466	IgG1
CD8-FITC	Miltenyi Biotec	BW/135/80	IgG2a
CD38-FITC	StemCell Technologies	AT-1	IgG1
CD80-FITC	BD Biosciences	L307.4	IgG1
CD86-PE	BD Biosciences	FUN-1	IgG1
CD123-FITC	BD Biosciences	7G3	IgG1
CD123-PE	BD Biosciences	7G3	IgG1
HLA-DR-FITC	BD Biosciences	G46-6	IgG2a

Absolute quantification of peripheral blood leukocyte sub-populations

The absolute numbers of all T cell subsets, myeloid dendritic cells and pDCs were determined using BD TruCount tubes as previously described.[14] Forward-scatter/side-scatter scattergrams were used to gate the PBMCs. The $CD3^+$ T cells, $CD3^+CD4^+$ T cells and $CD3^+CD4^-$ T cells were gated using CD3 and CD4 monoclonal antibodies (Figure 1). To determine numbers of myeloid dendritic cells and pDCs in populations of PBMCs, myeloid dendritic cells were phenotypically described as being $Lin^-HLA\text{-}DR^+CD11c^+$ cells and pDCs as being $HLA\text{-}DR^+CD123^+$ cells. The absolute numbers of the different PBMC subsets were determined using TruCount beads (BD Biosciences). Absolute cell numbers were calculated using the following formula: Cell concentration = (events in cells region) × (total count of beads in TruCount tube)/(events in beads region) × (sample volume).

Assay of IFN-α concentration by ELISA

Commercial ELISA kits (PBL Biomedical Laboratories, Piscataway, NJ, USA) were used to measure concentrations of IFN-α in the supernatant of stimulated PBMCs according to the manufacturers' protocol.

Statistical analysis

All the data were analyzed by an unpaired two-tailed Student's *t*-test using SPSS 16.0 software. For all of the statistical analyses, $P<0.05$ was considered significant.

RESULTS

Macaques in the late phase of SIVmac239 infection showed greater activation of pDCs and T lymphocytes

The macaques used in this study were all infected intravenously with SIVmac239. Three macaques (00067, 00079 and 01035) were considered to be in a later phase of SIVmac239 infection, having higher viral loads and lower $CD4^+$ T cell counts (lower than 500/μl), while the other three (04029, 04039 and 05049) were considered to be in a chronic phase of SIVmac239 infection, having lower viral loads and stable higher $CD4^+$ T cell counts. The SIV-negative macaques (06025, 06065 and 06067) were used as negative controls.

Reports have suggested that immune activation is critical for the pathogenesis of AIDS. Therefore, we tested the levels of immune activation in the three cohorts (Figure 1a and b). We found that the pDCs in the PBMCs from late-phase infected macaques had distinctly higher expression of CD80 and CD86 costimulatory molecules compared with macaques in that were chronically infected (Figure 1c and d), which indicated a higher pDC activation level. In addition, the level of activation of both the $CD4^+$ and $CD8^+$ T

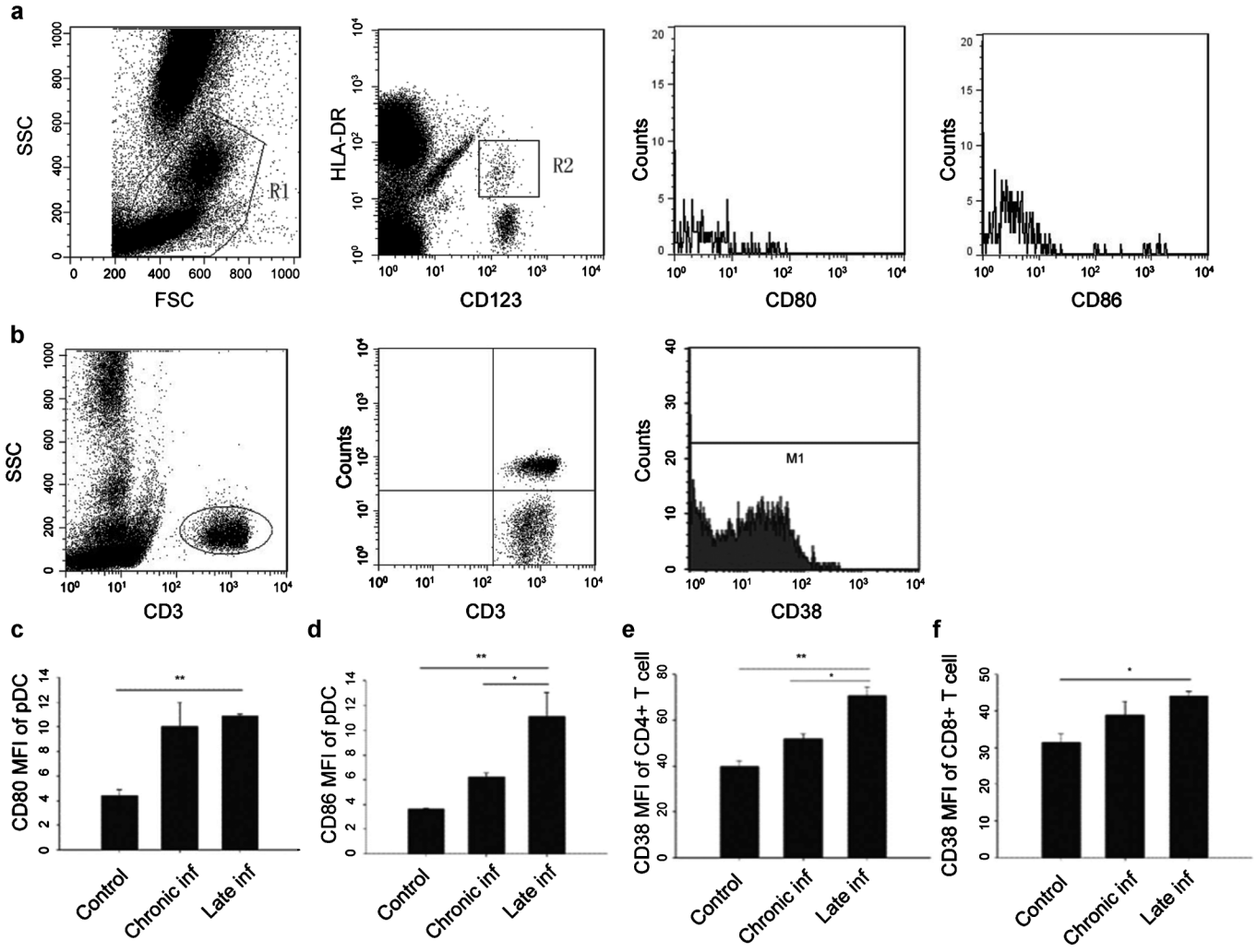

Figure 1 Immune activation levels in macaques in different phases of SIVmac239 infection. (**a**) PBMCs (R1) were first gated on FSC/SSC scattergrams. Then, $CD123^+HLA\text{-}DR^+$ cells (R2) were then selected as pDCs. The expression of the costimulating molecules CD80 and CD86 were shown in the histograms. In the T-cell analysis, the $CD3^+$ cells were gated as R1, then $CD3^+CD4^+$ (R2) and $CD3^+CD4^-$ (R3) T cells were selected to analyze the expression levels of CD38 (**b**). The macaques in the late phase of infection had more pDC (**c**, **d**) and T-lymphocyte (**e**, **f**) activation compared with the macaques that were chronically infected, while the SIV-negative macaques had no obvious immune activation. Graphs show the mean±s.d. $P<0.05$. FSC/SSC, forward-scatter/side-scatter; PBMC, peripheral blood mononuclear cell; pDC, plasmacytoid dendritic cell; s.d., standard deviation.

cells was elevated in the macaques in a later phase of infection (Figure 1e and f). All of the SIVmac239-infected macaques had higher levels of pDC and T-lymphocyte activation than the negative controls.

Chloroquine effectively inhibited TLR-induced pDC activation

pDCs express TLR7 and TLR9 within their endosomes. Binding of single-stranded RNAs or CpG to TLR7 or TLR9 in pDCs could lead to their activation. Activated pDCs might upregulate the expression of costimulating factors and the secretion of IFN-α. To determine whether chloroquine has the ability to inhibit the activation of pDCs through the TLR pathway, we used R837 (TLR7 ligand) and CpG2395 (TLR9 ligand) to stimulate the pDCs in PBMCs of Chinese rhesus macaques *in vitro* and tested the ability of chloroquine to inhibit the subsequent immune activation. We also used a TLR7 antagonist, ODN2088, as a positive control to evaluate the inhibition of the TLR pathway in pDCs. As shown in Figure 2b, R837 and CpG2395 could effectively activate pDCs *in vitro*, which was indicated by increased CD86 expression. Both TLR7- and TLR9-induced pDC activations were inhibited by chloroquine (Figure 2b).

To confirm the ability of chloroquine to inhibit immune activation in viral particle-induced pDC activation, we used concentrated SIVmac239 to stimulate PBMCs from SIV-negative Chinese rhesus macaques. Then, the expression levels of CD86 and IFN-α were then tested. As shown in Figure 2c and d, the pDCs were activated by SIVmac239 viral particles, and this activation was effectively inhibited by chloroquine. The levels of IFN-α in the culture supernatants also decreased during chloroquine treatment.

Because the macaques experienced a high viral load during SIVmac239 infection, plasma from these SIVmac239-infected primates may have the potential to stimulate pDCs and other immune cells through the TLR activation pathway. We treated PBMCs from healthy macaques with plasma from SIVmac239-infected macaques with or without chloroquine. We found that the plasma could activate the pDCs and that this activation was inhibited by the chloroquine (Figure 2e).

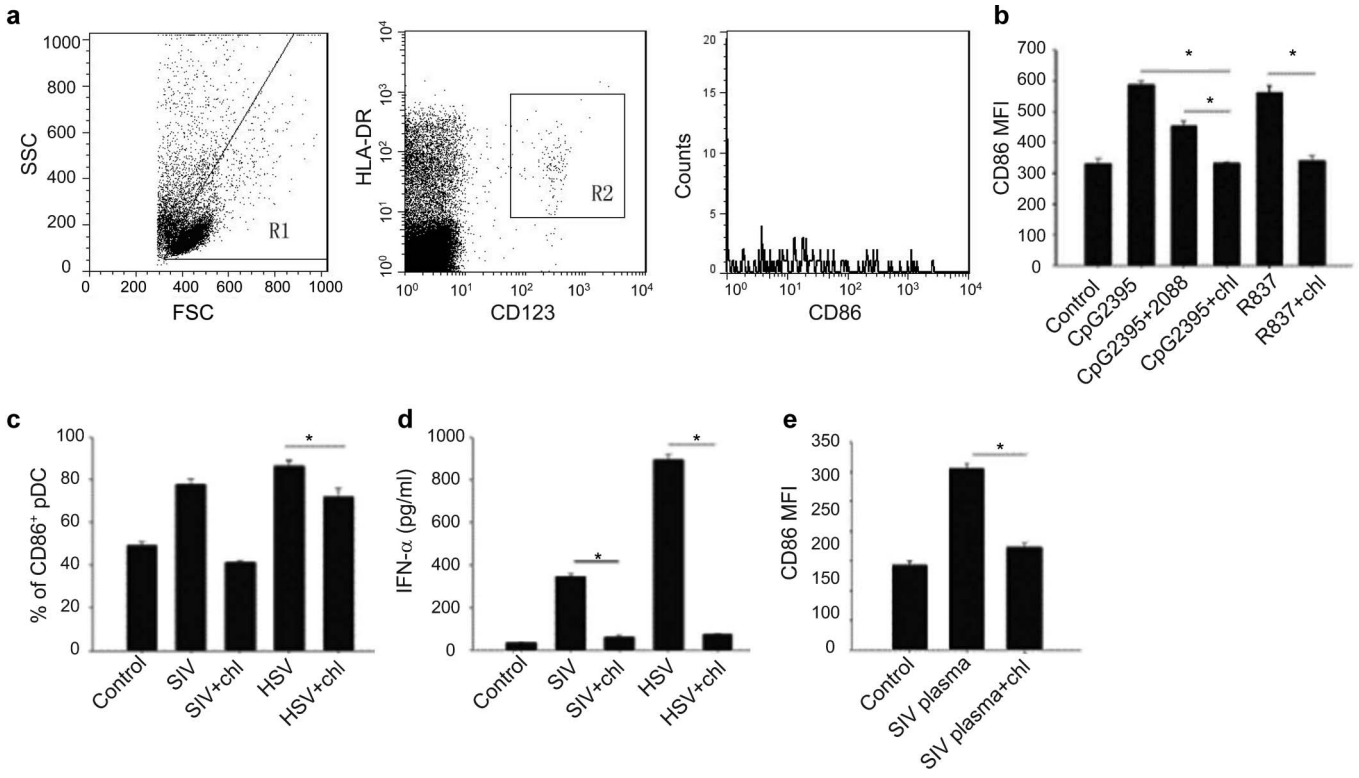

Figure 2 The inhibition of TLR-induced pDC activation by chloroquine treatment. The gating strategies shown in (**a**) were as mentioned in the Figure 1 legend. The inhibitory effects of chloroquine and ODN2088 on pDC activation were shown in (**b**). The SIVmac239 and HSV viral particles effectively activated the pDCs and this activation was inhibited by chloroquine treatment as shown by the expression of CD86 (**c**). The ability of chloroquine to inhibit viral particle-induced pDC activation was also demonstrated by the changes in IFN-α concentration in the supernatant (**d**). As shown in (**c**) and (**d**), the virions effectively activated the pDCs and this activation was inhibited by chloroquine treatment. The changes in pDC activation before and after the chloroquine treatment are shown in (**e**). Graphs show the mean±s.d. $P<0.05$. HSV, herpes simplex virus; IFN, interferon; pDC, plasmacytoid dendritic cell; s.d., standard deviation.

In vivo chloroquine inhibits pDC activation in SIVmac239-infected macaques

To test the ability of chloroquine to inhibit immune activation and its subsequent effect on disease progression *in vivo*, a solution of chloroquine was administered intragastrically to three macaques (00067, 01035 and 04029) infected with SIVmac239. At different time points, we tested the level of pDC and $CD4^+/CD8^+$ T-lymphocyte activation. Chloroquine was shown to effectively inhibit the activation of pDCs during SIV infection, as the expression levels of the costimulatory molecules CD80 and CD86 were reduced during treatment (Figure 3a and b). Most importantly, our results also showed an increase in pDC activation upon termination of the chloroquine treatment (Figure 3a and b). These results implicate the crucial role of chloroquine in the inhibition of pDC activation. However, there was no significant change in the level of T-lymphocyte activation with chloroquine treatment (Figure 3c and d).

To identify a possible relationship between the changes of blood cell composition and chloroquine treatment, we also tested the counts of different cell subsets in PBMC populations. As shown in Figure 3e–g, there were no obvious changes in the total counts of PBMCs, the different subsets of T lymphocytes or the pDCs.

DISCUSSION

Recently, persistent immune activation, which is described as elevated T-lymphocyte turnover and apoptosis; polyclonal B-cell activation; increased NK-cell activation and turnover; and dendritic cell activation,[7,14,15] was found to be a hallmark of HIV infection. In fact, it is well associated with $CD4^+$ T cell counts and HIV viral loads. It even correlates with $CD4^+$ T cell counts better than HIV RNA levels and could be used as an effective clinical prognostic indicator.[6] However, the molecular contributors to this immune activation in HIV/SIV infection remain unclear.

Brenchley *et al.*[16] suggested that microbial translation, which is a consequence of severe damage to the gastrointestinal tract during HIV infection, might play a critical role in chronic immune activation. They found elevated plasma lipopolysaccharide (LPS) levels in HIV-1-infected individuals, which served as an indicator of microbial translocation. Interestingly, these elevated levels were also shown to decrease during highly active antiretroviral therapy treatment (HAART). There was also evidence of an association between the LPS levels and adaptive immune activation. Studies of HIV-infected South Africans also suggested that microbial translocation might be a driving force of chronic inflammation.[5] Monocyte activity might play a major role in the cases of microbial translocation-induced immune activation.[5] It was reported that the level of monocyte turnover was more pronounced than the viral load and lymphocyte activation in SIV-infected rhesus macaques; this finding implied that monocytes may be very important in the pathogenesis of AIDS. As a result of high TLR4 and CD14 expression, monocytes in the plasma of HIV patients were sensitive to high levels of LPS and inflammatory cytokines;[17] this result implied that the role of LPS is important in the pathogenesis of AIDS.

Although this research supports microbial translocation as the cause of immune activation in HIV/SIV infections, there is also

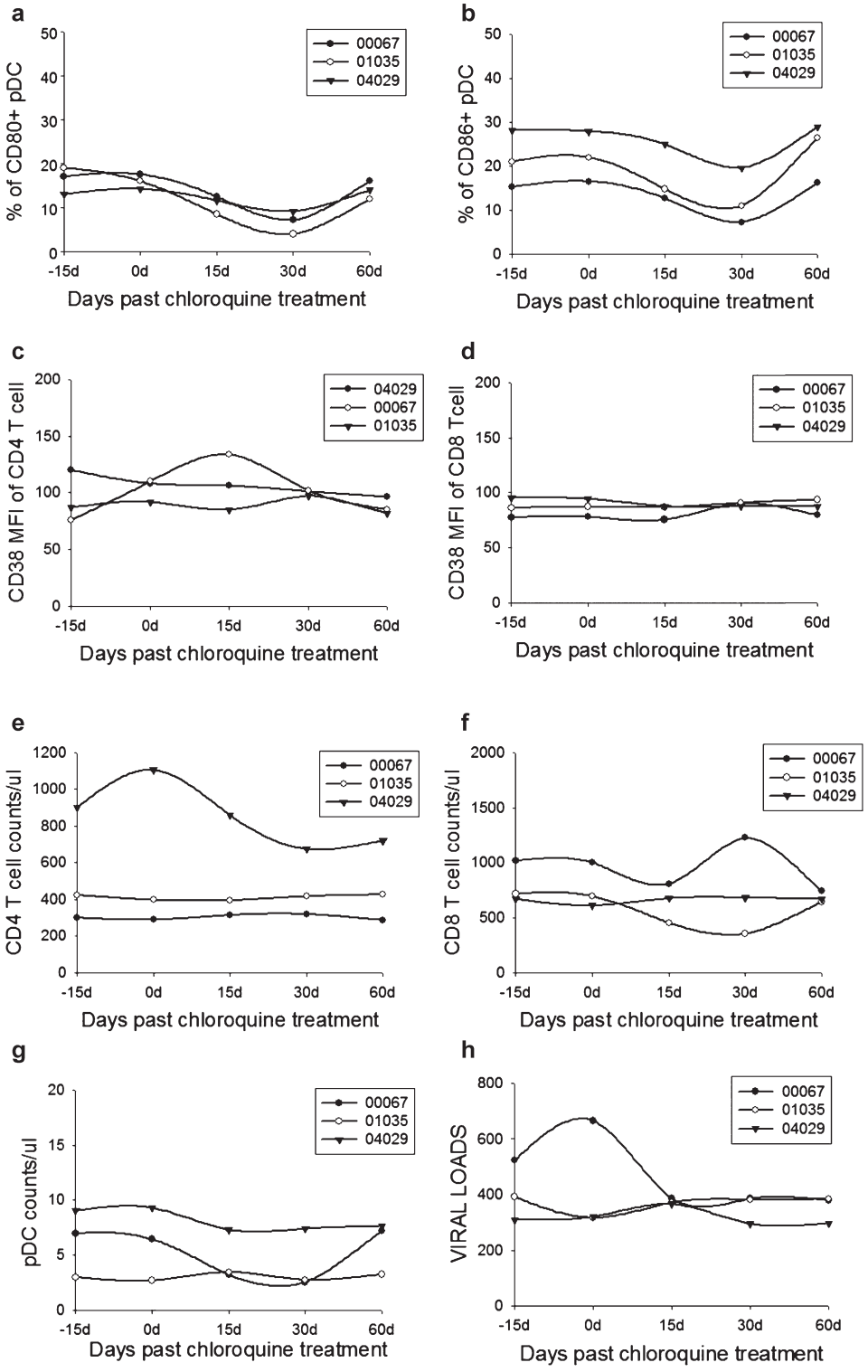

Figure 3 The influences of chloroquine treatment on SIVmac239-infected CRMs *in vivo*. As shown in (**a**) and (**b**), the level of pDC activation decreased during chloroquine treatment. However, there was no significant decrease in T-lymphocyte activation in this study (**c**, **d**). During chloroquine treatment, the numbers of CD4 T cells (**e**), CD8 T cells (**f**) and pDCs (**g**) and plasma viral load (**h**) in the peripheral blood did not change. CRM, Chinese rhesus macaque; pDC, plasmacytoid dendritic cell; SIV, simian immunodeficiency virus.

evidence that virions might directly participate in the process of immune activation through pDC activation.[10]

pDCs are a subset of dendritic cells that play a very critical role in the immune response against pathogens. Not only are they the main source of type I IFNs but they also serve to bridge the gap between innate and adaptive immunity.[18–20] Like other cells that participate in the immune response, pDCs express many pathogen recognition receptors to sense the conserved molecular products of pathogens. For example, the single-stranded RNA sensor TLR7 and the CpG region sensor TLR9 are both expressed on the endosomes of pDCs.[21] During HIV infection, the HIV virions enter into the pDC endosome through endocytosis. Upon degradation of the virions' capsid, interaction of virion RNA with TLR7 could cause pDC activation. A previous study in our lab also showed that the expression levels of costimulatory molecules (CD80, CD83 and CD86) and chemokine receptors (CCR5 and CCR7) were both upregulated during the acute SIVmac239 infection of Chinese rhesus macaques. These expression levels were also correlated with plasma viral loads, which implies that the viruses might cause pDC activation during SIV infection.[22]

Many reports suggest that increased levels of type I IFNs, which are caused by TLR stimulation, might be very important in AIDS pathogenesis, although the quick type I IFN response in the early phase of infection effectively controlled viral replication.[10] pDCs produce large quantities of type I IFNs, especially IFN-α, upon activation by a pathogen.[20] The IFN-α receptor is widely expressed by many cell types. Some subtypes of IFN-α might even be important in immunomodulation rather than having direct antiviral activity. These facts indicate that high levels of IFN-α could have a great deal of influence on immune cells.[20] Therefore, type I IFN levels are likely to play a role in the pathogenesis of AIDS during HIV or SIV infection.[23]

Meier *et al.*[24] reported the difference in the rate of AIDS progression between male and female HIV-positive patients correlated with level of IFN-α, as the PBMCs derived from female patients produced higher levels of IFN-α in response to TLR7 ligands than those derived from male patients. It was also reported that circulating pDCs in HIV-infected individuals showed an increase in IFN-α expression.[25]

Baenziger *et al.*[26] found an AIDS-like pathology in mice treated with TLR7 ligands. African green monkeys, which are considered to be the natural host of SIVagm, will not progress to AIDS even though they show high viral loads upon SIV infection. Jacquelin *et al.*[27] reported a strong type I IFN response in the acute phase of infection in the African green monkey, but immune activation was controlled during chronic infection, a response that differed from that of the rhesus macaques. In addition, our study also showed that pDC activation is highly correlated with disease pathogenesis in SIVmac239 infection, as the macaques in the late phase of infection had higher pDC activation compared with the chronic infected macaques.

Considering the important role played by pDCs and IFN-α, effective methods of inhibiting pDC activation might be crucial to AIDS treatment. As the RNA of HIV could activate pDCs through TLR7 stimulation and this process requires the acidic environment of the endosome where TLRs that sense viral RNA are expressed, strategies to block this process could effectively inhibit pDC activation.

Chloroquine, which is a 9-aminoquinoline that has been known to have antimalarial activity for more than half a century, was recently found to have some new potential uses.[8] It was reported that chloroquine could directly inhibit viral replication through endosomal pH changes, as an acidic environment is crucial for viral replication. Chloroquine could disrupt the endosomal TLR pathway by inhibiting the acidification of the endosome, which is crucial for pDC activation and subsequent IFN-α secretion.[10] Consequently, it is believed that chloroquine could have a potential use in the inhibition of immune activation during HIV infection. Martison *et al.*[9] suggested that chloroquine could effectively inhibit HIV virion-induced pDC and T-lymphocyte activation *in vitro*. Murray *et al.*[11,12] found that there was a reduction in immune activation in chloroquine-treated chronic HIV-1-infected individuals. However, there are no related studies on the non-human primate AIDS models, which are very important in AIDS pathology research and novel treatment evaluation.

In this study, we found that SIVmac239-infected macaques that were in a relatively later phase of infection, showed greater pDC and T-lymphocyte activation than chronically infected macaques; this finding suggests that immune activation may be related to disease progression. We also found that chloroquine could effectively inhibit pDC activation induced by TLR ligands or SIV virions *in vitro*. As expected, chloroquine also reduced the level of pDC activation in SIVmac239-infected Chinese rhesus macaques *in vivo*. These results imply the potential use of chloroquine as an effective inhibitor of the endosomal TLR pathway. However, the effect of chloroquine had no observable influence on the cell composition of peripheral blood. The short duration of treatment might have had an effect on the results in this study and should be considered when planning future studies. Our results in this study also implied that other factors, such as LPS, might participate in chronic immune activation as well. Consequently, the single effect of decreasing pDC activation did not have an obvious impact on the activation of T lymphocytes. As a result, the mechanisms of immune activation in HIV infection and the potential role of chloroquine as an anti-AIDS drug will require further research.

ACKNOWLEDGEMENTS

This work was supported in part by grants from the National Basic Research Program of China (2009CB522306 and 2012CBA01305), the National Natural Science Foundation of China (30872317, 30800113, U0832601 and 81172876), the Knowledge Innovation Program of CAS (KSCX1-YW-10 and KSCX2-EW-R-13), the Key Scientific and Technological Program of China (2009ZX09501-029, 2012ZX10001-006 and 2012ZX10001-007) and Yunnan province (2010GA001) and the 'Western Light' Projects of the CAS. The funding organizations had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

1 Ketloy C, Engering A, Srichairatanakul U, Limsalakpetch A, Yongvanitchit K, Pichyangkul S *et al*. Expression and function of Toll-like receptors on dendritic cells and other antigen presenting cells from non-human primates. *Vet Immunol Immunopathol* 2008; **125**: 18–30.
2 John L, Lutwama F. A review of the use of activation markers in Africa. *J HIV Ther* 2010; **15**: 11–14.
3 Mir KD, Gasper MA, Sundaravaradan V, Sodora DL. SIV infection in natural hosts: resolution of immune activation during the acute-to-chronic transition phase. *Microbes Infect* 2011; **13**: 14–24.
4 Deeks SG, Kitchen CM, Liu L, Guo H, Gascon R, Narváez AB *et al*. Immune activation set point during early HIV infection predicts subsequent $CD4^+$ T-cell changes independent of viral load. *Blood* 2004; **104**: 942–947.
5 Hazenberg MD, Otto SA, van Benthem BH, Roos MT, Coutinho RA, Lange JM *et al*. Persistent immune activation in HIV-1 infection is associated with progression to AIDS. *AIDS* 2003; **17**: 1881–1888.
6 Kovacs A, Karim R, Mack WJ, Xu J, Chen Z, Operskalski E *et al*. Activation of CD8 T cells predicts progression of HIV infection in women coinfected with hepatitis C virus. *J Infect Dis* 2010; **201**: 823–834.
7 Chang JJ, Altfeld M. Innate immune activation in primary HIV-1 infection. *J Infect Dis* 2010; **202** (Suppl 2):S297–S301.
8 Savarino A, Boelaert JR, Cassone A, Majori G, Cauda R. Effects of chloroquine on viral infections: an old drug against today's diseases? *Lancet Infect Dis* 2003; **11**: 722–727.
9 Martinson JA, Montoya CJ, Usuga X, Ronquillo R, Landay AL, Desai SN. Chloroquine modulates HIV-1-induced plasmacytoid dendritic cell alpha interferon: implication for T-cell activation. *Antimicrob Agents Chemother* 2010; **54**: 871–881.

10 Beignon AS, McKenna K, Skoberne M, Manches O, DaSilva I, Kavanagh DG *et al*. Endocytosis of HIV-1 activates plasmacytoid dendritic cells *via* Toll-like receptor-viral RNA interactions. *J Clin Invest* 2005; **11**: 3265–3275.
11 Murray SM, Down CM, Boulware DR, Stauffer WM, Cavert WP, Schacker TW *et al*. Reduction of immune activation with chloroquine therapy during chronic HIV infection. *J Virol* 2010; **22**: 12082–12086.
12 Piconi S, Parisotto S, Rizzardini G, Passerini S, Terzi R, Argenteri B *et al*. Hydroxychloroquine drastically reduces immune activation in HIV-infected, antiretroviral therapy-treated immunologic nonresponders. *Blood* 2011; **12**: 3263–3272.
13 Xia HJ, Zhang GH, Ma JP, Dai ZX, Li SY, Han JB *et al*. Dendritic cell subsets dynamics and cytokine production in SIVmac239-infected Chinese rhesus macaques. *Retrovirology* 2010; **7**: 102.
14 Xia HJ, Zhang GH, Wang RR, Zheng YT. The influence of age and sex on the cell counts of peripheral blood leukocyte subpopulations in Chinese rhesus macaques. *Cell Mol Immunol* 2009; **6**: 433–440.
15 Lane HC, Masur H, Edgar LC, Whalen G, Rook AH, Fauci AS. Abnormalities of B-cell activation and immunoregulation in patients with the acquired immunodeficiency syndrome. *N Engl J Med* 1983; **309**: 453–458.
16 Brenchley JM, Price DA, Schacker TW, Asher TE, Silvestri G, Rao S *et al*. Microbial translocation is a cause of systemic immune. *Nat Med* 2006; **12**: 1365–1371.
17 Said EA, Dupuy FP, Trautmann L, Zhang Y, Shi Y, El-Far M *et al*. Programmed death-1-induced interleukin-10 production by monocytes impairs $CD4^+$ T cell activation during HIV infection. *Nat Med* 2010; **16**: 452–459.
18 Colonna M, Trinchieri G, Liu YJ. Plasmacytoid dendritic cells in immunity. *Nat Immunol* 2004; **5**: 1219–1226.
19 Zhang Z, Wang FS. Plasmacytoid dendritic cells act as the most competent cell type in linking antiviral innate and adaptive immune responses. *Cell Mol Immunol* 2005; **6**: 411–417.
20 Fitzgerald-Bocarsly P, Dai J, Singh S. Plasmacytoid dendritic cells and type I IFN: 50 year of convergent history. *Cytokine Growth Factor Rev* 2008; **19**: 3–19.
21 Iwasaki A, Medzhitov R. Toll-like receptor control of the adaptive immune responses. *Nat Immunol* 2004; **5**: 987–995.
22 Xia HJ, Ma JP, Zhang GH, Han JB, Wang JH, Zheng YT. Effect of plasma viremia on apoptosis and immunophenotype of dendritic cells subsets in acute SIVmac239 infection of Chinese rhesus macaques. *PLoS ONE* 2011; **6**: e29036.
23 Herbeuval JP, Shearer GM. HIV-1 immunopathogenesis: how good interferon turns bad. *Clin Immunol* 2007; **123**: 121–128.
24 Meier A, Chang JJ, Chan ES, Pollard RB, Sidhu HK, Kulkarni S *et al*. Sex differences in the Toll-like receptor-mediated response of plasmacytoid dendritic cells to HIV-1. *Nat Med* 2009; **8**: 955–959.
25 Lehmann C, Harper JM, Taubert D, Hartmann P, Fätkenheuer G, Jung N *et al*. Increased interferon alpha expression in circulating plasmacytoid dendritic cells of HIV-1-infected patients. *J Acquir Immune Defic Syndr* 2008; **48**: 522–530.
26 Baenziger S, Heikenwalder M, Johansen P, Schlaepfer E, Hofer U, Miller RC *et al*. Triggering TLR7 in mice induces immune activation and lymphoid system disruption, resembling HIV-mediated pathology. *Blood* 2009; **113**: 377–388.
27 Jacquelin B, Mayau V, Targat B, Liovat AS, Kunkel D, Petitjean G *et al*. Nonpathogenic SIV infection of African green monkeys induces a strong but rapidly controlled type I IFN response. *J Clin Invest* 2009; **12**: 3544–3555.