Early Divergence in Lymphoid Tissue Apoptosis between Pathogenic and Nonpathogenic Simian Immunodeficiency Virus Infections of Nonhuman Primates

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Early divergence in lymphoid tissue apoptosis between pathogenic and non-pathogenic SIV infections of non-human primates.

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* This work is dedicated to Bruno Hurtrel

Abstract

The events that contribute to the progression to AIDS during the acute phase of a primate lentiviral infection are still poorly understood. In this study, we have used pathogenic and non-pathogenic simian models of SIV infection of rhesus macaque (RM) and African green monkeys (AGM), respectively, to investigate the relationship between apoptosis in lymph nodes and its relationship with the extent of viral replication, immune activation and disease outcome.

Here we show that, in SIVmac251-infected RMs, a marked increased in lymphocyte apoptosis is evident during primary infection at the level of lymph nodes. Interestingly, the levels of apoptosis correlated with the extent of viral replication and the rate of disease progression to AIDS, with higher apoptosis in RMs of Indian genetic background when compared to those of Chinese origin. In stark contrast, no changes in the levels of lymphocyte apoptosis were observed during primary infection in the non-pathogenic model of SIVagm-sab infection of AGMs despite similarly high viral replication. A further and early divergence between SIV-infected RMs and AGMs was observed in terms of dynamics of T and B cell proliferation in lymph nodes, with RMs showing significantly higher levels of $Ki67^+$ cells in the T cell zones in association with relatively low levels of $Ki67^+$ in the B cell zones, whereas AGMs displayed a low frequency of $Ki67^+$ in the T cell area but a high proportion of $Ki67^+$ cells in the B cell area. As such, this study suggests that species-specific host factors determine an early immune response to SIV that is predominantly involves either cellular or humoral immunity in RMs and AGMs, respectively.

Taken together, these data are consistent with the hypotheses that: (i) high levels of T cell activation and lymphocyte apoptosis are key pathogenic factor during pathogenic SIV infection of RMs, and (ii) low T cell activation and apoptosis are determinants of the AIDS-resistance of SIVagm-infected AGMs despite high levels of SIVagm replication.

Introduction

While rhesus macaques (RMs) infected with simian immunodeficiency virus (SIVmac) usually progress to AIDS in 1-2 years, African non-human primates (NHPs) infected with their species-specific SIV rarely develop disease. Previous studies of natural, non-pathogenic primate models of SIV infection, such as SIVagm infection of African green monkeys (AGMs), SIVsmm or SIVmac infection of sooty mangabeys (SMs), and SIVmnd-1 and SIVmnd-2 infection of mandrills (MNDs) have consistently shown that, in these animals, the levels of plasma viral load are similar to those observed in human immunodeficiency virus (HIV)-infected humans and SIVmac-infected rhesus macaques (RMs) (2, 6, 10, 16, 18, 19, 29, 32, 35, 39). However, only HIV-infections in humans and SIVmac infections in RMs lead to progressive $CD4^+$ T-cell depletion and AIDS. Understanding the basis of pathogenic and nonpathogenic host-virus relationships is likely to provide important clues regarding AIDS pathogenesis.

The primary acute phase of HIV and SIV infections is characterized by an early burst of viral replication, an exponential increase in viral load, the dissemination and seeding of the virus in all peripheral lymphoid organs, a severe depletion of memory $CD4^+$ T cells from the mucosal-associated lymphoid tissue (MALT), and the induction of the host immune response against the virus (21, 24, 27, 41, 45). Several observations indicate that the early induction of an effective immune response against the virus plays a role in determining levels of viral load at the end of the primary phase (i.e., set-point). The levels of viral load in peripheral blood is a strong predictor of disease progression in pathogenic lentiviral infection (21, 31, 36, 37, 45). Moreover, increased level of immune activation in pathogenic lentiviral infection is correlated with disease progression towards AIDS (5, 27, 39). Importantly, the early stages of non-pathogenic SIV infection of AGMs and SMs are also characterized by a peak of virus replication in peripheral blood accompanied by rapid dissemination of the virus and depletion of $CD4^+$ T-cells from the MALT (19, 32). However, in these non-progressing hosts, it has been shown that the level of the immune activation remains relatively low when compared to RMs (2, 8, 19, 32, 40).

An increased lymphocyte susceptibility to apoptosis, that is in turn though to be related to heightened levels of immune activation, has been proposed as one of the main

mechanisms responsible for the $CD4^+$ T-cell depletion *in vivo* during pathogenic HIV and SIV infections (1, 12, 17). Studies performed in pathogenic and non-pathogenic primate models of SIV-infection during the asymptomatic phase have further suggested the presence of a direct correlation between progression to AIDS and the level of $CD4^+$ T cell apoptosis *ex vivo* (8, 40, 44). We recently reported that primary SIVmac251 infection of RMs of Chinese origin, that are known to progress to AIDS more slowly than Indian monkeys (22, 34), is also associated with increased numbers of apoptotic cells in the lymph nodes (25, 43). The reasons why the course of SIVmac infection is milder in Chinese RMs (as compared to Indian RMs) are still poorly understood. Since SIVmac strains were propagated either *in vivo* or *in vitro* in cells derived from Indian monkeys, this may have resulted in the selection of viral variants that are adapted for more efficient replication and/or increased pathogenicity for these animals (34).

Here we reports the results of a comparative study of the initial interactions between SIV and the host immune system in two different species of non-human primates (NHP) that exhibit a divergent outcome of the infection, i.e., non-natural RM hosts, where the infection leads to $CD4^+$ T cell depletion and AIDS, and natural AGMs hosts, where the infection is typically non-pathogenic. To investigate the potential immunopathogenic role of lymphocyte apoptosis in lymph nodes occurring during the primary phase of infection, we performed a longitudinal study of 20 experimentally SIV-infected NHPs (6 Indian RMs and 8 Chinese RMs infected with SIVmac251; and 6 African Green Monkeys infected with SIVagm-sab). We examined the frequency of (i) SIV RNA^+ cells, (ii) apoptotic cells ($TUNEL^+$), (iii) TiA-1-positive cells and (iv) cycling $Ki67^+$ cells in this diverse array of pathogenic and nonpathogenic primate models that are associated with peculiar rates of progression to AIDS (or lack thereof). Our data demonstrate that the early levels of apoptosis and immune activation are key events associated with progression to AIDS.

Animals and virus infection

The following animals were include in this study: (i) Fourteen RMs of either Indian (n=6) or Chinese (n=8) origin were intravenously inoculated with ten 50% animal infectious doses (AID_{50}) of the pathogenic SIVmac251; and (ii) Six AGMs of sabaeus species were experimentally infected with 300 TCID50 of SIVagm.sab92018 strain. As previously described, this virus derives from a naturally infected AGM and has never been cultured *in vitro* (6). The pathogenic SIVmac251 isolate was initially provided by R. Desrosiers and titrated in Chinese rhesus macaques (*Macaca mulatta*) by intravenous inoculation. A 1-ml volume of stock virus contained 4 x 10^4 50% animal infectious doses (AID_{50}) as previously described (25). Animals were demonstrated as being seronegative for STLV-1 (Simian T Leukemia Virus type-1), SRV-1 (type D retrovirus), herpes-B viruses, and SIVmac. Animals were housed and cared for in compliance with existing French regulations (http://www.pasteur.fr/recherche/unites/animalerie/fichiers/Decret2001-486.pdf).

Determination of viral load and quantitative assessment of productively infected cells

RNA was extracted from plasma of SIV-infected monkeys, using the TRI REAGENT BD Kit (Molecular Research Center Inc., Cincinnati, Ohio). Real-time quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) was used to determine viral loads as previously described (25). The inguinal and axillary lymph nodes (LNs) were sequentially collected from each animal during the course of primary infection. The LNs were frozen in isopentane cooled in liquid nitrogen and then cryostat sectioned at 4 µm intervals. Viral replication in LNs was assessed by *in situ* hybridization using a ^{35}S-labeled RNA probe as previously described (25). Infected cells, detected as spots, were counted in the paracortical zone on a minimum of three sections using a Nikon-FXA microscope. The number of positive cells (counted as spots) was then divided by the surface of the entire LN section, and results were expressed as the number of positive cells per 2mm^2 section. The mean count obtained for three slides of the same LNs, in a blinded fashion, by two investigators, was calculated.

Quantitative assessment of apoptotic cells

Apoptotic cells were identified using the TUNEL assay, which detects DNA fragmentation usually associated with apoptosis as previously described (25). Briefly, cryostat tissue sections were fixed in 2% paraformaldehyde for 10 min, and overlaid with TUNEL reaction mixture containing terminal deoxyribonucleotidyl transferase (Boerhinger Mannheim, Mannheim, Germany) and Dig-deoxyuridine triphosphate. The sections were incubated for 1

h at 37°C. The sections of LN were washed in phosphate-buffered saline and incubated with a sheep anti-Dig-alcaline phosphatase antibody for 40 min at 37°C and revealed with naphthol AS-MX, fast red, levamisol. LN sections were counterstained using Harris haematoxylin and mounted with gelatin. The number of positive cells in the T-cell areas was then divided by the surface of the entire LN section, and results were expressed as the number of positive cells per $2mm^2$ section. The mean count obtained for three slides of the same LNs, in a blinded fashion, by two investigators, was calculated.

Quantitative assessment of cycling cells

Cycling cells were assessed using a monoclonal antibody to the Ki67 nuclear proliferation-associated antigen (MIB-1; Immunotech, Inc., Marseille, France) that is expressed at all phases of the cell cycle except the G0 phase. Alcaline phosphatase was used as the chromogen, and the slides were slightly counterstained with haematoxylin as previously described (25). The numbers of LN-positive cells counted in the T-cell areas were then divided by the surface of the entire LN section, and results were expressed as positive cell numbers/2 mm^2 section. The proportion of cycling cells (%GCKi67%) in the B-cell areas (Germinal Centers, GCs) was quantified and divided by the surface of the entire LN section. The proportion obtained for three slides of the same LNs, in a blinded fashion, by two investigators, was calculated.

Quantitative assessment of TiA1 cells

TiA1 cells were assessed using a monoclonal antibody (clone GMP-17, Beckman Coulter). Alcaline phosphatase was used as the chromogen, and the slides were slightly counterstained with haematoxylin. The proportion of TiA1-positive cells counted in the T-cell areas were then divided by the surface of the entire LN section, and results were expressed as %. These analyses were performed on four different sections, in a blind fashion, by two investigators.

Lymphocyte immunophenotyping by flow cytometry

T-cells were stained with the following fluorochrome-labeled monoclonal antibodies: anti-rhesus monkey CD3-conjugated fluorescein-isothiocyanate (FITC) (clone FN18, Biosource International), anti-human CD4 conjugated with phycoerythrin (PE) (clone M-T477, BD Biosciences), anti-human CD8-conjugated with peridinin-chlorophyll protein (PerCp) (BD Biosciences) and anti–human leukocyte antigen (HLA)-DR-conjugated with allophycocyanin (APC) (L243, BD Biosciences). Antibodies were added to 100 µl of $2x10^5$ LN cells. Cells

were incubated for 15 min at room temperature. The cells were then washed once in PBA buffer (phosphate-buffered saline-1% bovine serum albumin-10 mM NaN3) and resuspended in PBS containing 1% paraformaldehyde (PBS-PF) as previously described (reference).

Statistical analyses

The Student's t tests and Mann-Whitney U-test (Prism software) were used to determine whether differences were significant if $p<0.05$. Mann-Whitney U-test was used to compare results between monkey species. Spearman's rank correlation, implemented from Statistica software, was use to evaluate correlations. Best-fit lines are shown.

196 **Results**

197

198 1) Viral replication and disease outcomes

199 Rhesus macaques (RMs) of Indian genetic background progressed more rapidly
200 towards AIDS than Chinese RMs despite receiving similar viral stock of SIVmac251 strain
201 that was produced in PBMCs of Chinese monkeys. In this study, all Indians RMs were
202 sacrificed due to the onset of AIDS-related symptoms before one year of infection, while half
203 of the SIV-infected Chinese RMs was alive after 36 months of infection. As expected, all the
204 SIV-infected AGMs were alive at >48 months post infection (**Figure 1A**).

205

206 During acute SIV infection, the initial burst in viral replication causes the exponential
207 increase in plasma viral load that leads to the dissemination of the virus throughout the body
208 and the infection of the $CD4^+$ T cells in the MALT (42) as well as in peripheral lymphoid
209 organs, which are the main sites of both virus replication and immune response to the virus.
210 As shown in **Figure 1B**, we first assessed the dynamics of viral load in these different groups
211 of SIV-infected NHPs. In RMs, viremia peaked between days 11 and 14 with values ranging
212 between $7.5x10^6$ and $7x10^7$ copies/ml in RMs of Indian origin and between $6.7x10^5$ and
213 $3.2x10^7$ copies/ml in Chinese RMs. In AGMs, viremia peaked between days 8 and 14 and the
214 values ranged between $7.9x10^6$ and $1.2x10^8$ copies/ml. The set-point levels in AGMs ranged
215 between $1.3x10^3$ and $2.5x10^5$ copies/ml whereas in Indian RMs and Chinese RMs it ranged
216 between $3x10^4$ and $1.8x10^7$ copies/ml and $3x10^2$ and $5.7x10^5$ copies/ml, respectively. Thus,
217 these data are consistent with numerous previous observations (13, 19, 21, 25, 27, 32, 41, 45)
218 in indicating that both peak and set-point viremia are similar in pathogenic and non-
219 pathogenic primate models**.**

220

221 To longitudinally assess the relationship between the level of the early viral
222 dissemination/seeding and the immune system dynamics (i.e., proliferation and cell death),
223 we sequentially collected the inguinal and axillary lymph nodes (LNs) from each animal
224 during the course of primary infection. In all examined tissues, SIV RNA^+ cells were
225 promptly detected, in the pathogenic as well as in the non-pathogenic models (**Figure 2A**).
226 During the acute phase of SIVmac251 infection of RMs, the peak numbers of productively
227 infected cells per surface unit in the LN T-cell area was reached at days 11-14 post-infection,
228 i.e., coincident with the peak of viral loads, and was then followed by a significant decrease
229 by day 60 (**Figure 2B**). Interestingly, the level of viral replication in LN was significantly

higher in RMs of Indian origin when compared to Chinese RMs, with peak numbers of productively infected cells of 204.8 ± 125 SIV RNA$^+$ cells/2mm^2 and 30.8 ± 19.7 SIV RNA$^+$ cells/2mm^2, respectively (P=0.007, using Mann-Whitney U-test) (**Figure 2B**). Similarly, the frequency of CD4$^+$ T cells harboring proviral DNA at the peak values was higher in Indian than in Chinese RMs (0.043 ± 0.038 and 0.017 ± 0.009, respectively, data not shown). This difference persisted at day 60, with the numbers of SIV RNA$^+$ cells/2mm^2 higher in Indian than in Chinese RMs (14.2 ± 11.2 and 7.9 ± 8.8, respectively). Most importantly, we found that the numbers of SIV RNA$^+$ cells/2mm^2 in AGMs at the peak of virus replication (21 ± 12.5) were relatively similar to those observed at the peak in SIVmac251-infected Chinese RMs (**Figure 2B**). However, it should be noted that, at day 60, the number of SIV RNA$^+$ cells/2mm^2 in LNs was extremely low in AGMs (0.53 ± 0.22), despite similar levels of viral loads in these two species.

These findings suggest that the level of peak viral replication in the LNs is not discriminative of pathogenic and non-pathogenic primate models of SIV infection (with Indian RMs showing, nevertheless, higher frequencies of infected cells and faster disease progression than Chinese RMs). Interestingly, the level of viral replication in peripheral LNs during the set-point phase of SIV infection was higher in SIVmac251-infected RMs than in SIVagm-sab-infected AGMs, thus suggesting that other anatomic sites (i.e., MALT, spleen etc) are responsible for the high plasma viremia of AGMs.

2) Lymph node apoptosis is a typical feature of pathogenic SIV-infections

We then sought to determine, in the same groups of SIV-infected NHPs, the extent of apoptotic cells in LNs. The numbers of apoptotic cells were quantified in the T-cell areas of tissue sections from the same LN in which numbers of SIV-infected cells were previously assessed **(Figure 3A)**. We found that the number of apoptotic cells increased after infection and reached their highest levels at the time of peak viral replication in both Indian and Chinese RMs (Day 14, 138 ± 66 cells/2mm^2 and 77 ± 16 cells/2mm^2, respectively, P = 0.04 using Mann-Whitney U-test). The numbers of apoptotic cells remained higher at day 60 (Indian, 58 ± 59 cells/2mm^2 *versus* Chinese, 16 ± 7 cells/mm^2; P = 0.04) than before infection (8 ± 6 cells/mm^2) **(Figure 3B)**. Importantly, a significant direct correlation was found in SIVmac251-infected RMs between the level of viral replication within LNs (as measured at the time of peak viremia) and the numbers of apoptotic cells **(Figure 3C)**. In SIVagm-infected AGMs the level of T-cell apoptosis in the LNs remained similar to baseline

throughout the acute phase of infection (Day 13, 25 ± 28 cells/2mm^2 and day 60, 27 ± 9 cells/2mm^2). The observation that T cell apoptosis does not increase in AGMs during early SIV infection despite levels of viral replication that are similar to those observed in SIVmac251-infected Chinese RMs suggests that indirect (i.e., non virus-related) mechanisms rather than the direct effect of viral-replication are responsible for the increased rate of apoptotic cells in RMs.

3) Dynamics of cycling cells in the T cell area

To investigate the relationship between the extent of LN apoptosis and the level of immune activation (that results in frequent events of activation-induced cell death (17)), we first assessed the level of activated (Ki67$^+$) lymphocytes in our group of animals. We found that, in both Indian and Chinese SIVmac251-infected RMs, the paracortical areas (T-cell zone) contained more activated cells than those of uninfected RMs **(Figure 4)**. The number of cycling cells peaked at days 11-14 post-infection (Day 14, 2537 ± 1154 cells/2mm^2 and 2430 ± 1130 cells/2mm^2 in Indian and Chinese RMs, respectively) and remained much higher than baseline at day 60 (1655 ± 1099 cells/2mm^2 and 1626 ± 502 cells/2mm^2, in Indian and Chinese RMs, respectively, versus Day 0, 45 ± 25 cells/2mm^2) **(Figure 4B)**. Of note, the differences in Ki67$^+$ cells between Indian and Chinese RMs were not statistically significant, thus suggesting that the higher level of apoptotic cells observed during primary SIVmac251 infection of Indian RMs is not simply a consequence of greater numbers of cycling T cells.

We next measured the numbers of cycling lymphocytes in SIVagm-infected AGMs, and found that cycling cells peaked at day 8 (739 ± 173 cells/2mm^2 versus day 0, 133 ± 103 cells/2mm^2, P = 0.002) with a slight decrease thereafter (Day 60, 596 ± 110 cells/2mm^2) which was coincident with the decline of viral replication in LNs **(Figure 4B)**. While SIV-infected AGMs show a clear increase in the numbers of cycling T cells when compared to baseline (i.e., prior to infection), the absolute levels of T cell activation in the LNs of these animals are approximately one third lower of those observed in SIVmac251-infected RMs (**Figure 4B**). Therefore, this result is consistent with previous reports indicating that non-pathogenic SIV infection of natural hosts is associated with lower levels of immune activation (8, 40).

To further characterize the dynamics of T cell activation in our group of NHPs, we next assessed by flow cytometry the changes in the levels of CD3$^+$CD4$^+$ and CD3$^+$CD8$^+$ T

cells expressing HLA-DR in peripheral blood. Interestingly, SIVmac251-infected Indian RMs revealed a profound decrease in the percentage of $CD4^+DR^+$ T cells, while only a transient decline of $CD4^+DR^+$ T cells was observed at the peak of virus replication in Chinese monkeys **(Figure 5)**. Moreover, we found an early increase in the fraction of $CD8^+DR^+$ T cells in Chinese RMs that still persists at the time of set-point viremia **(Figure 5)**. In SIV-infected AGMs, we found a transitory increase in the activation status of $CD8^+$ T cells (but not $CD4^+$ T cells), with levels of $CD8^+DR^+$ T cells peaking at day 14 but soon returning to levels similar to those observed prior to infection. This pattern of T cell activation in SIVagm-infected AGMs is very similar to that previously reported by our and other laboratories (19, 32).

While our data failed to identify any significant correlation between the extent of T cell activation and the rate of apoptosis in pathogenic SIVmac251 infection of RMs, it was clearly apparent that, in the non-pathogenic SIVagm infection of AGMs, an attenuated level of T cell activation was associated with lower levels of apoptosis.

4) Dynamics of cycling cells in the B cell area

We next assessed the level of activated ($Ki67^+$) cells in the B-cell zone by staining the germinal centers (GC). In contrast to the large increase of cycling cells in the T cell area observed in SIVmac251-infected RMs, no changes in the level of $Ki67^+GC^+$ (% $Ki67^+GC^+$) was found in the same animals at the peak of viral replication. Interestingly, the level of B-cell activation within the GC was three- to five-fold higher in SIV-infected AGMs than in RMs at day 60, with 29.3% ± 9.7 of $Ki67^+GC^+$ in AGMs, 11.2% ± 8.1 in Chinese RMs, 6.2% ± 3.1 in Indian RMs ($P = 0.008$), and 0.155 % ± 0.25 in uninfected animals **(Figure 4C)**. We also found that the percentage of $CD20^+CD3^-$ cells (B cells), as analyzed by flow cytometry at day 60 p.i., was increased in LNs of SIV-infected AGMs (28.3% ± 3.5) when compared to preinfection values (15.2% ± 1.7). These dynamics are consistent with the time of detection of SIV-specific antibodies in the plasma that appear between days 30 and 60 (data not shown), and suggest that immune responses to SIV involve predominantly the humoral immunity in AGMs.

4) Dynamics of TiA-1+ cells

To further characterize the early activation of $CD8^+$ T cells observed during SIV infection of RMs and AGMs, we examined the expression of cytolytic granules by staining for TiA-1, i.e., a polyadenylate binding protein localized to the granules of cytolytic T cells

that is functionally related to perforin. In these experiments we assessed TiA-1 expression *in situ* to exclude the possibility that isolated lymphocytes might release their granule contents during isolation procedure **(Figure 6)**. We found that, in the LNs of SIVagm-infected AGMs, the fraction of TiA-1 expressing cells showed an early moderate increase (Day 8, 0.5% ± 0.3 *versus* Day 0, 0.13% ± 0.1; P = 0.018) followed by a slight decline by the time of set-point viremia (Day 60, 0.25% ± 0.2) **(Figure 6B)**. As such, the dynamics of TiA-1 expression in AGMs followed the dynamics observed for the proportion of activated $CD8^+$ T-cells.

In SIVmac251-infected RMs, the frequency of TiA-1 expressing T-cells increased earlier in Indian animals (as compared to Chinese). However, at day 60 post-infection the level of TiA-1 reactivity in SIVmac251-infected Chinese RMs was greater than before infection (6.5% ± 1.5 versus 1.9% ± 0.39; P = 0.006) and greater than in Indian RMs (3.9% ± 1.6; P=0.02) **(Figure 6B)**. This dynamics of TiA-1 expression in LNs during SIVmac251 infection of RMs was consistent with the observed pattern of $CD8^+DR^+$ T cell dynamics.

Discussion

In this study, we report the results of a detailed longitudinal study of a large number (n=20) of non-human primates experimentally infected with SIV, including 6 Indian and 8 Chinese RMs infected with SIVmac251, and 6 AGMs infected with SIVagm-sab. The key goal of this study was to assess, in the different non-human primate models of infection, the relationship between virus replication, immune activation, lymphocyte apoptosis, and disease progression. A key premise of this study is the observation that, in striking contrast to HIV-infected humans and SIVmac-infected RMs, natural SIV hosts, such as AGMs, SMs, and mandrills, remain typically asymptomatic despite similarly high levels of virus replication (2, 6, 8, 14, 15, 19, 29, 32, 40). It is now widely accepted that understanding the mechanisms underlying the different infection outcomes in natural versus non-natural SIV hosts will provide important clues as to the mechanisms of AIDS pathogenesis.

SIVmac infection of Chinese RMs is less pathogenic than that of Indian RMs and thus more closely mimics the course of HIV infection in humans (5, 26, 27). As some of this earlier experiments were conducted using virus preparations obtained through repeated serial passage in cells from Indian RMs (or in Indian RMs *in vivo*), it was proposed that increased viral fitness from Indian RM cells may explain the higher replicative capacity and faster disease progression in these animals. However, in our experiments, we used a SIVmac251

strain that has been *in vitro* passaged in PBMC derived from Chinese RMs. We still observe an accelerated course of disease in Indian RMs, thus suggesting that host determinants, rather than intrinsic properties of SIV are key determinants of the divergent infection outcomes in RMs.

Our study also confirmed and extended previous evaluations of viral dynamics in peripheral blood demonstrating that levels of viral replication in RMs and AGMs are quite similar. Importantly, by the end of the acute phase of infection, a clear difference emerged between AGMs and RMs in term of viral replication in LNs, with numbers of SIV RNA^+ cells that were significantly lower in SIV-infected AGMs compared with those observed in RMs (Indian RMs being higher than Chinese RMs). These data indicate that the level of virus replication in the LNs at the end of the acute phase may be a good predictor of disease progression in RMs (25-27), and that, in AGMs, the level of viral particles in the blood likely reflects virus replication occurring in sites other than peripheral LNs, such as the MALT or spleen.

The data included in the current study strongly suggests that the extent of apoptotic cell death occurring in the T-cell areas of the peripheral lymph nodes represents an early pathogenic event that seems to determine the subsequent course of disease progression. The fact that SIVagm-infected AGMs do not display any major increase in T-cell apoptosis in the context of significant levels of viral replication suggests that the direct effects of SIV replication alone are unlikely to account for the high levels of T cell apoptosis observed in RMs at the time of peak viremia. Instead, our observation is consistent with a model whereby large numbers of T cells undergo apoptosis as a consequence of a massive event of immune activation. Interestingly, in RMs, we found a positive correlation between the level of viral replication in the LNs and the extent of T cell apoptosis, suggesting either a role, at least partial, for viral replication-mediated cell death in these animals or, alternatively, a role for SIV antigens in determining—through the generation of virus-specific immune responses—the prevailing levels of immune activation and apoptosis. In other studies we have shown that the extent of apoptosis in peripheral LNs was greater in Chinese RMs infected with pathogenic SIVmac than in those infected with a *nef*-deleted SIVmac strain and predicted, in these animals, the risk of progression to AIDS (25, 43). Altogether, these results suggest that in SIV-infected RMs, increased levels of apoptosis represent a key determinant of the progressive immunodeficiency, thus consistent with previous reports performed during the

chronic phase of HIV and SIV infection (8, 9, 12, 17, 26, 27). In this context, it is tempting to speculate that an additional deleterious effect of the increased T-cell death observed in SIVmac251-infected RMs is the inability of the immune system to mount a sustained immune response to the virus (43).

Altogether, these findings suggest that the level of lymphocyte apoptosis in the early stages of SIV infection is a species-specific surrogate marker of AIDS pathogenesis. In this study it was clearly apparent that, in the non-pathogenic SIVagm infection of AGMs, an attenuated T cell activation was associated with lower levels of apoptosis during the acute phase. This new finding is consistent with earlier reports indicating during the chronic phase of SIV infection a relationship between the extent of immune activation, T cell apoptosis and pathogenesis (8, 40). This absence of immune activation in SIV-infected AGMs might be related to an early anti-inflammatory profile characterized by the induction of Treg cells (19). Interestingly, SIV-infected AGMs showed LNs of lower size than RMs (data not shown), thus further suggesting that the "total body" level of immune activation is significantly lower during non-pathogenic infections of natural SIV hosts.

The increase of TiA1-expressing T cells in LNs concomitant with the observation of a transient increase of activated $CD8^+$ T cells (DR^+) during the acute viral replication period in AGMs is consistent with recent reports (13, 32). This observation supports the hypothesis that the post-peak decline in virus replication observed in the LNs of SIVagm-infected AGMs might be, at least in part, related to the emergence of cellular immune responses to the virus. On the other hand, however, it should be noted that, to this date, there is no experimental evidence indicating that in SIV-infected AGMs (or any other natural SIV hosts), the cellular immune responses to the virus are broader or more potent/effective that those described in pathogenic HIV-1 and SIV infections (7). Alternatively, the observed low levels of activated $CD4^+$ T cells (DR^+) in SIV-infected AGMs may suggest that the post-peak decrease of viral load in the LNs of SIVagm-infected AGMs is related to the rapid exhaustion of target cells due to the attenuated levels of local T cell activation.

Interestingly, we found a clear difference in the dynamics of T and B cells between AGMs and RMs. SIVmac251-infected RMs showed more extensive and persistent proliferation of T cells than SIVagm-infected AGMs. On the contrary, SIVagm-infected AGMs showed a more prominent B cell activation than SIVmac251-infected RMs, as

manifested by the level of Ki67$^+$ cells in the GCs at the set point than RMs. This observation is also consistent with two recent reports indicating that SIV infection of AGMs is associated with high levels of Ki67+ cells in the GCs and rapid expansion of B cells (13, 32). While these results seem at odd with the observation that the titer of SIV-specific antibodies are lower in AGMs than RMs (28), it should be noted that high fractions of Ki67$^+$ GC cells do not necessarily translate in high titers of SIV-specific antibody. In any event, our intriguing observation of an early and selective B cell response during non-pathogenic SIVagm infection of AGMs suggest that the role of SIV-specific humoral responses in primate lentiviral infections should be further explored in future studies, particularly with respect to the impact of SIV-specific antibodies in the control and/or compartmentalization of viral replication at the end of the acute phase.

A question that remains to be answered is how host genetic factors may influence the strikingly different level of lymphocyte apoptosis in LNs that we observed during early SIV infection in the various NHP models studied. A first possibility is that individual differences in the signaling pathways that participate in immune activation and apoptosis regulation may lead to different sensitivity to death signal transduction induced either by viral proteins or by death ligands and receptors (such as CD95 ligand and CD95) (9, 11). It has been recently proposed a role for CD95/CD95L in the depletion of uninfected CD4$^+$ T cells occurring during primary infection in the lamina propria of SIV-infected RMs (20). As the extent of T-cell activation is typically lower in AGMs than in RMs, species-specific differences in the immunomodulatory activity of Treg cells might be beneficial in controlling T-cell activation and activation-induced cell death (AICD) (19). A second possibility is that differences in the induction of apoptosis are related to quantitative differences in the initial levels of co-receptors expression. Several studies have suggested that co-receptor expression affect disease progression in HIV-infected individuals (4, 23, 38) as the proportion of CD4$^+$CCR5$^+$ and/or the density of cell-surface CCR5 correlate with disease progression (3, 30). Moreover, it has been recently reported a difference in the level of CCR5 between pathogenic and non-pathogenic primate models of AIDS (33). Finally, we recently demonstrated that in addition to CCR5, ligation of GPR15 (BOB), an additional co-receptor utilized by the SIVmac251 strain, mediates CD4$^+$ T cell death (43). However, dynamics of CD4$^+$ T cells expressing this latter co-receptor are still unknown.

In conclusion, the current set of data is clearly consistent with the hypothesis that the prevailing levels of immune activation and T cell apoptosis are key marker of disease progression during pathogenic primate lentiviral infections. Similarly, this work supports the concept that limited immune activation and low levels of T cell apoptosis are instrumental to avoid progression to AIDS in AGMs and other natural SIV hosts as well.

References

1. **Arnoult, D., F. Petit, J. D. Lelievre, D. Lecossier, A. Hance, V. Monceaux, B. Hurtrel, R. Ho Tsong Fang, J. C. Ameisen, and J. Estaquier.** 2003. Caspase-dependent and -independent T-cell death pathways in pathogenic simian immunodeficiency virus infection: relationship to disease progression. Cell Death Differ **10:**1240-52.
2. **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. J Virol **75:**2262-75.
3. **de Roda Husman, A. M., H. Blaak, M. Brouwer, and H. Schuitemaker.** 1999. CC chemokine receptor 5 cell-surface expression in relation to CC chemokine receptor 5 genotype and the clinical course of HIV-1 infection. J Immunol **163:**4597-603.
4. **Dean, M., M. Carrington, C. Winkler, G. A. Huttley, M. W. Smith, R. Allikmets, J. J. Goedert, S. P. Buchbinder, E. Vittinghoff, E. Gomperts, S. Donfield, D. Vlahov, R. Kaslow, A. Saah, C. Rinaldo, R. Detels, and S. J. O'Brien.** 1996. Genetic restriction of HIV-1 infection and progression to AIDS by a deletion allele of the CKR5 structural gene. Hemophilia Growth and Development Study, Multicenter AIDS Cohort Study, Multicenter Hemophilia Cohort Study, San Francisco City Cohort, ALIVE Study. Science **273:**1856-62.
5. **Deeks, S. G., C. M. Kitchen, L. Liu, H. Guo, R. Gascon, A. B. Narvaez, P. Hunt, J. N. Martin, J. O. Kahn, J. Levy, M. S. McGrath, and F. M. Hecht.** 2004. Immune activation set point during early HIV infection predicts subsequent CD4+ T-cell changes independent of viral load. Blood **104:**942-7.
6. **Diop, O. M., A. Gueye, M. Dias-Tavares, C. Kornfeld, A. Faye, P. Ave, M. Huerre, S. Corbet, F. Barre-Sinoussi, and M. C. Muller-Trutwin.** 2000. High levels of viral replication during primary simian immunodeficiency virus SIVagm

infection are rapidly and strongly controlled in African green monkeys. J Virol **74:**7538-47.

7. **Dunham, R., P. Pagliardini, S. Gordon, B. Sumpter, J. Engram, A. Moanna, M. Paiardini, J. N. Mandl, B. Lawson, S. Garg, H. M. McClure, Y. X. Xu, C. Ibegbu, K. Easley, N. Katz, I. Pandrea, C. Apetrei, D. L. Sodora, S. I. Staprans, M. B. Feinberg, and G. Silvestri.** 2006. The AIDS resistance of naturally SIV-infected sooty mangabeys is independent of cellular immunity to the virus. Blood **108:**209-17.

8. **Estaquier, J., T. Idziorek, F. de Bels, F. Barre-Sinoussi, B. Hurtrel, A. M. Aubertin, A. Venet, M. Mehtali, E. Muchmore, P. Michel, and et al.** 1994. Programmed cell death and AIDS: significance of T-cell apoptosis in pathogenic and nonpathogenic primate lentiviral infections. Proc Natl Acad Sci U S A **91:**9431-5.

9. **Estaquier, J., T. Idziorek, W. Zou, D. Emilie, C. M. Farber, J. M. Bourez, and J. C. Ameisen.** 1995. T helper type 1/T helper type 2 cytokines and T cell death: preventive effect of interleukin 12 on activation-induced and CD95 (FAS/APO-1)-mediated apoptosis of CD4+ T cells from human immunodeficiency virus-infected persons. J Exp Med **182:**1759-67.

10. **Estaquier, J., V. Monceaux, M. C. Cumont, A. M. Aubertin, B. Hurtrel, and J. C. Ameisen.** 2000. Early changes in peripheral blood T cells during primary infection of rhesus macaques with a pathogenic SIV. J Med Primatol **29:**127-35.

11. **Estaquier, J., M. Tanaka, T. Suda, S. Nagata, P. Golstein, and J. C. Ameisen.** 1996. Fas-mediated apoptosis of CD4+ and CD8+ T cells from human immunodeficiency virus-infected persons: differential in vitro preventive effect of cytokines and protease antagonists. Blood **87:**4959-66.

12. **Finkel, T. H., G. Tudor-Williams, N. K. Banda, M. F. Cotton, T. Curiel, C. Monks, T. W. Baba, R. M. Ruprecht, and A. Kupfer.** 1995. Apoptosis occurs predominantly in bystander cells and not in productively infected cells of HIV- and SIV-infected lymph nodes. Nat Med **1:**129-34.

13. **Goldstein, S., C. R. Brown, I. Ourmanov, I. Pandrea, A. Buckler-White, C. Erb, J. S. Nandi, G. J. Foster, P. Autissier, J. E. Schmitz, and V. M. Hirsch.** 2006. Comparison of simian immunodeficiency virus SIVagmVer replication and CD4+ T-cell dynamics in vervet and sabaeus African green monkeys. J Virol **80:**4868-77.

14. **Goldstein, S., I. Ourmanov, C. R. Brown, B. E. Beer, W. R. Elkins, R. Plishka, A. Buckler-White, and V. M. Hirsch.** 2000. Wide range of viral load in healthy african

green monkeys naturally infected with simian immunodeficiency virus. J Virol **74:**11744-53.

15. **Gueye, A., O. M. Diop, M. J. Ploquin, C. Kornfeld, A. Faye, M. C. Cumont, B. Hurtrel, F. Barre-Sinoussi, and M. C. Muller-Trutwin.** 2004. Viral load in tissues during the early and chronic phase of non-pathogenic SIVagm infection. J Med Primatol **33:**83-97.

16. **Holzammer, S., E. Holznagel, A. Kaul, R. Kurth, and S. Norley.** 2001. High virus loads in naturally and experimentally SIVagm-infected African green monkeys. Virology **283:**324-31.

17. **Hurtrel, B., F. Petit, D. Arnoult, M. Muller-Trutwin, G. Silvestri, and J. Estaquier.** 2005. Apoptosis in SIV infection. Cell Death Differ **12 Suppl 1:**979-90.

18. **Kaur, A., R. M. Grant, R. E. Means, H. McClure, M. Feinberg, and R. P. Johnson.** 1998. Diverse host responses and outcomes following simian immunodeficiency virus SIVmac239 infection in sooty mangabeys and rhesus macaques. J Virol **72:**9597-611.

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. J Clin Invest **115:**1082-91.

20. **Li, Q., L. Duan, J. D. Estes, Z. M. Ma, T. Rourke, Y. Wang, C. Reilly, J. Carlis, C. J. Miller, and A. T. Haase.** 2005. Peak SIV replication in resting memory CD4+ T cells depletes gut lamina propria CD4+ T cells. Nature **434:**1148-52.

21. **Lifson, J. D., M. A. Nowak, S. Goldstein, J. L. Rossio, A. Kinter, G. Vasquez, T. A. Wiltrout, C. Brown, D. Schneider, L. Wahl, A. L. Lloyd, J. Williams, W. R. Elkins, A. S. Fauci, and V. M. Hirsch.** 1997. The extent of early viral replication is a critical determinant of the natural history of simian immunodeficiency virus infection. J Virol **71:**9508-14.

22. **Ling, B., R. S. Veazey, A. Luckay, C. Penedo, K. Xu, J. D. Lifson, and P. A. Marx.** 2002. SIV(mac) pathogenesis in rhesus macaques of Chinese and Indian origin compared with primary HIV infections in humans. Aids **16:**1489-96.

23. **Liu, R., W. A. Paxton, S. Choe, D. Ceradini, S. R. Martin, R. Horuk, M. E. MacDonald, H. Stuhlmann, R. A. Koup, and N. R. Landau.** 1996. Homozygous

defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. Cell **86:**367-77.

24. **Mellors, J. W., C. R. Rinaldo, Jr., P. Gupta, R. M. White, J. A. Todd, and L. A. Kingsley.** 1996. Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. Science **272:**1167-70.

25. **Monceaux, V., J. Estaquier, M. Fevrier, M. C. Cumont, Y. Riviere, A. M. Aubertin, J. C. Ameisen, and B. Hurtrel.** 2003. Extensive apoptosis in lymphoid organs during primary SIV infection predicts rapid progression towards AIDS. Aids **17:**1585-96.

26. **Monceaux, V., R. Ho Tsong Fang, M. C. Cumont, B. Hurtrel, and J. Estaquier.** 2003. Distinct cycling CD4(+)- and CD8(+)-T-cell profiles during the asymptomatic phase of simian immunodeficiency virus SIVmac251 infection in rhesus macaques. J Virol **77:**10047-59.

27. **Monceaux, V., L. Viollet, F. Petit, R. Ho Tsong Fang, M. C. Cumont, J. Zaunders, B. Hurtrel, and J. Estaquier.** 2005. CD8+ T cell dynamics during primary simian immunodeficiency virus infection in macaques: relationship of effector cell differentiation with the extent of viral replication. J Immunol **174:**6898-908.

28. **Norley, S. G., G. Kraus, J. Ennen, J. Bonilla, H. Konig, and R. Kurth.** 1990. Immunological studies of the basis for the apathogenicity of simian immunodeficiency virus from African green monkeys. Proc Natl Acad Sci U S A **87:**9067-71.

29. **Onanga, R., C. Kornfeld, I. Pandrea, J. Estaquier, S. Souquiere, P. Rouquet, V. P. Mavoungou, O. Bourry, S. M'Boup, F. Barre-Sinoussi, F. Simon, C. Apetrei, P. Roques, and M. C. Muller-Trutwin.** 2002. High levels of viral replication contrast with only transient changes in CD4(+) and CD8(+) cell numbers during the early phase of experimental infection with simian immunodeficiency virus SIVmnd-1 in Mandrillus sphinx. J Virol **76:**10256-63.

30. **Ostrowski, M. A., S. J. Justement, A. Catanzaro, C. A. Hallahan, L. A. Ehler, S. B. Mizell, P. N. Kumar, J. A. Mican, T. W. Chun, and A. S. Fauci.** 1998. Expression of chemokine receptors CXCR4 and CCR5 in HIV-1-infected and uninfected individuals. J Immunol **161:**3195-201.

31. **Oxenius, A., D. A. Price, P. J. Easterbrook, C. A. O'Callaghan, A. D. Kelleher, J. A. Whelan, G. Sontag, A. K. Sewell, and R. E. Phillips.** 2000. Early highly active

antiretroviral therapy for acute HIV-1 infection preserves immune function of CD8+ and CD4+ T lymphocytes. Proc Natl Acad Sci U S A **97:**3382-7.

32. **Pandrea, I., C. Apetrei, J. Dufour, N. Dillon, J. Barbercheck, M. Metzger, B. Jacquelin, R. Bohm, P. A. Marx, F. Barre-Sinoussi, V. M. Hirsch, M. C. Muller-Trutwin, A. A. Lackner, and R. S. Veazey.** 2006. Simian immunodeficiency virus SIVagm.sab infection of Caribbean African green monkeys: a new model for the study of SIV pathogenesis in natural hosts. J Virol **80:**4858-67.

33. **Pandrea, I., C. Apetrei, S. N. Gordon, J. Barbercheck, J. Dufour, R. Bohm, B. S. Sumpter, P. Roques, P. A. Marx, V. M. Hirsch, A. Kaur, A. A. Lackner, R. S. Veazey, and G. Silvestri.** 2006. Paucity of CD4+CCR5+ T-cells is a typical feature of natural SIV hosts. Blood **109**: 1069-76.

34. **Reimann, K. A., R. A. Parker, M. S. Seaman, K. Beaudry, M. Beddall, L. Peterson, K. C. Williams, R. S. Veazey, D. C. Montefiori, J. R. Mascola, G. J. Nabel, and N. L. Letvin.** 2005. Pathogenicity of simian-human immunodeficiency virus SHIV-89.6P and SIVmac is attenuated in cynomolgus macaques and associated with early T-lymphocyte responses. J Virol **79:**8878-85.

35. **Rey-Cuille, M. A., J. L. Berthier, M. C. Bomsel-Demontoy, Y. Chaduc, L. Montagnier, A. G. Hovanessian, and L. A. Chakrabarti.** 1998. Simian immunodeficiency virus replicates to high levels in sooty mangabeys without inducing disease. J Virol **72:**3872-86.

36. **Rosenberg, E. S., M. Altfeld, S. H. Poon, M. N. Phillips, B. M. Wilkes, R. L. Eldridge, G. K. Robbins, R. T. D'Aquila, P. J. Goulder, and B. D. Walker.** 2000. Immune control of HIV-1 after early treatment of acute infection. Nature **407:**523-6.

37. **Rosenberg, E. S., J. M. Billingsley, A. M. Caliendo, S. L. Boswell, P. E. Sax, S. A. Kalams, and B. D. Walker.** 1997. Vigorous HIV-1-specific CD4+ T cell responses associated with control of viremia. Science **278:**1447-50.

38. **Samson, M., F. Libert, B. J. Doranz, J. Rucker, C. Liesnard, C. M. Farber, S. Saragosti, C. Lapoumeroulie, J. Cognaux, C. Forceille, G. Muyldermans, C. Verhofstede, G. Burtonboy, M. Georges, T. Imai, S. Rana, Y. Yi, R. J. Smyth, R. G. Collman, R. W. Doms, G. Vassart, and M. Parmentier.** 1996. Resistance to HIV-1 infection in caucasian individuals bearing mutant alleles of the CCR-5 chemokine receptor gene. Nature **382:**722-5.

39. **Silvestri, G., A. Fedanov, S. Germon, N. Kozyr, W. J. Kaiser, D. A. Garber, H. McClure, M. B. Feinberg, and S. I. Staprans.** 2005. Divergent host responses

635 during primary simian immunodeficiency virus SIVsm infection of natural sooty
636 mangabey and nonnatural rhesus macaque hosts. J Virol **79:**4043-54.
637 40. **Silvestri, G., D. L. Sodora, R. A. Koup, M. Paiardini, S. P. O'Neil, H. M.**
638 **McClure, S. I. Staprans, and M. B. Feinberg.** 2003. Nonpathogenic SIV infection of
639 sooty mangabeys is characterized by limited bystander immunopathology despite
640 chronic high-level viremia. Immunity **18:**441-52.
641 41. **Staprans, S. I., P. J. Dailey, A. Rosenthal, C. Horton, R. M. Grant, N. Lerche,**
642 **and M. B. Feinberg.** 1999. Simian immunodeficiency virus disease course is
643 predicted by the extent of virus replication during primary infection. J Virol **73:**4829-
644 39.
645 42. **Veazey, R. S., M. DeMaria, L. V. Chalifoux, D. E. Shvetz, D. R. Pauley, H. L.**
646 **Knight, M. Rosenzweig, R. P. Johnson, R. C. Desrosiers, and A. A. Lackner.**
647 1998. Gastrointestinal tract as a major site of CD4+ T cell depletion and viral
648 replication in SIV infection. Science **280:**427-31.
649 43. **Viollet, L., V. Monceaux, F. Petit, R. H. Fang, M. C. Cumont, B. Hurtrel, and J.**
650 **Estaquier.** 2006. Death of CD4+ T cells from lymph nodes during primary
651 SIVmac251 infection predicts the rate of AIDS progression. J Immunol **177:**6685-94.
652 44. **Wallace, M., P. M. Waterman, J. L. Mitchen, M. Djavani, C. Brown, P. Trivedi,**
653 **D. Horejsh, M. Dykhuizen, M. Kitabwalla, and C. D. Pauza.** 1999. Lymphocyte
654 activation during acute simian/human immunodeficiency virus SHIV(89.6PD)
655 infection in macaques. J Virol **73:**10236-44.
656 45. **Watson, A., J. Ranchalis, B. Travis, J. McClure, W. Sutton, P. R. Johnson, S. L.**
657 **Hu, and N. L. Haigwood.** 1997. Plasma viremia in macaques infected with simian
658 immunodeficiency virus: plasma viral load early in infection predicts survival. J Virol
659 **71:**284-90.
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Legends:

Figure 1: (A) Month survival. The percentage of survival in three different groups of SIV-infected monkeys (Indian rhesus macaques (RMs) and Chinese RMs infected with SIVmac251; AGMs infected with SIVagm.sab92018) during the follow-up period of this study is shown. Clinical progression towards disease was continuously evaluated. The animals were killed for a wasting syndrome with cachexia and opportunistic infections. (B) Viremia. Kinetic analysis of viremia in SIV-infected Indian and Chinese RMs, and AGMs.

Figure 2: Productive SIV infection in LNs from Indian and Chinese SIVmac251-infected RMs, and SIVagm-infected AGMs at different time point post-infection. (A) SIV RNA^+ cells were detected in LNs of SIV-infected monkeys (magnification, x100). (B) Quantitative assessment of SIV RNA^+ cells. Statistical significance was assessed using paired Student t test.

Figure 3: Immunohistochemical analysis of apoptotic cells in LNs from Indian and Chinese SIVmac251-infected RMs, and SIVagm-infected AGM at different time points after infection. (A) Apoptotic cells measured by Tunel method (magnification, x100). (B) Quantitative assessment of apoptotic cells. Statistical significance was assessed using paired Student t test. (C) Correlation between the extent of viral replication (LogSIV RNA^+) and the extent of apoptosis at the peak. Each symbol represents one individual RMs either from Indian (❍) or Chinese (●) RMs.

Figure 4: Immunohistochemical analyses of cycling cells in LNs from Indian and Chinese SIVmac251-infected RMs, and SIV-infected AGMs at different time points post-infection. (A) Ki67 staining was performed in LNs of SIV-infected monkeys (magnification, x100). (B) Quantitative assessment of Ki67 in T-cell area, and (C) B-cell area (%$GCKi67^+$). Statistical significance was assessed using paired Student t test.

Figure 5: Dynamics of HLA-DR on $CD4^+$ and $CD8^+$ T cells in LNs. Flow cytometry was used to quantify the percentage of T cells expressing HLA-DR gating on $CD3^+$ cells of Indian and Chinese SIVmac251-infected RMs, and SIV-infected AGMs at different time points post-infection. Data shown were calculated as follow: $DR^+CD4/CD8^+$ /($DR^+CD4/CD8^+$ + $DR^-CD4/CD8^+$) x 100. Statistical significance was assessed using paired Student t test.

696 Figure 6: Immunohistochemical analyses of TiA-1 from Indian and Chinese SIVmac251-
697 infected RMs, and SIV-infected AGMs at different time point post-infection. (A) TiA-1
698 staining was performed in LNs of SIV-infected monkeys (magnification, x100). (B)
699 Quantitative assessment of TiA-1 cells. Statistical significance was assessed using paired
700 Student t test.
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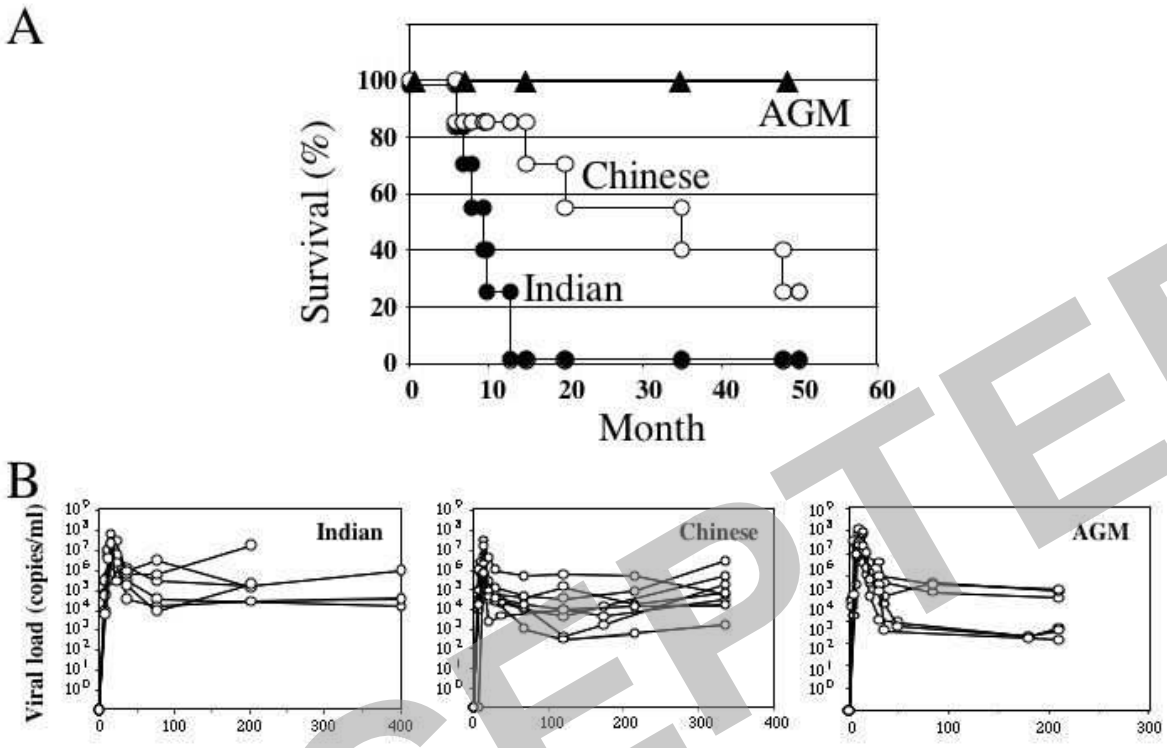

Figure 1

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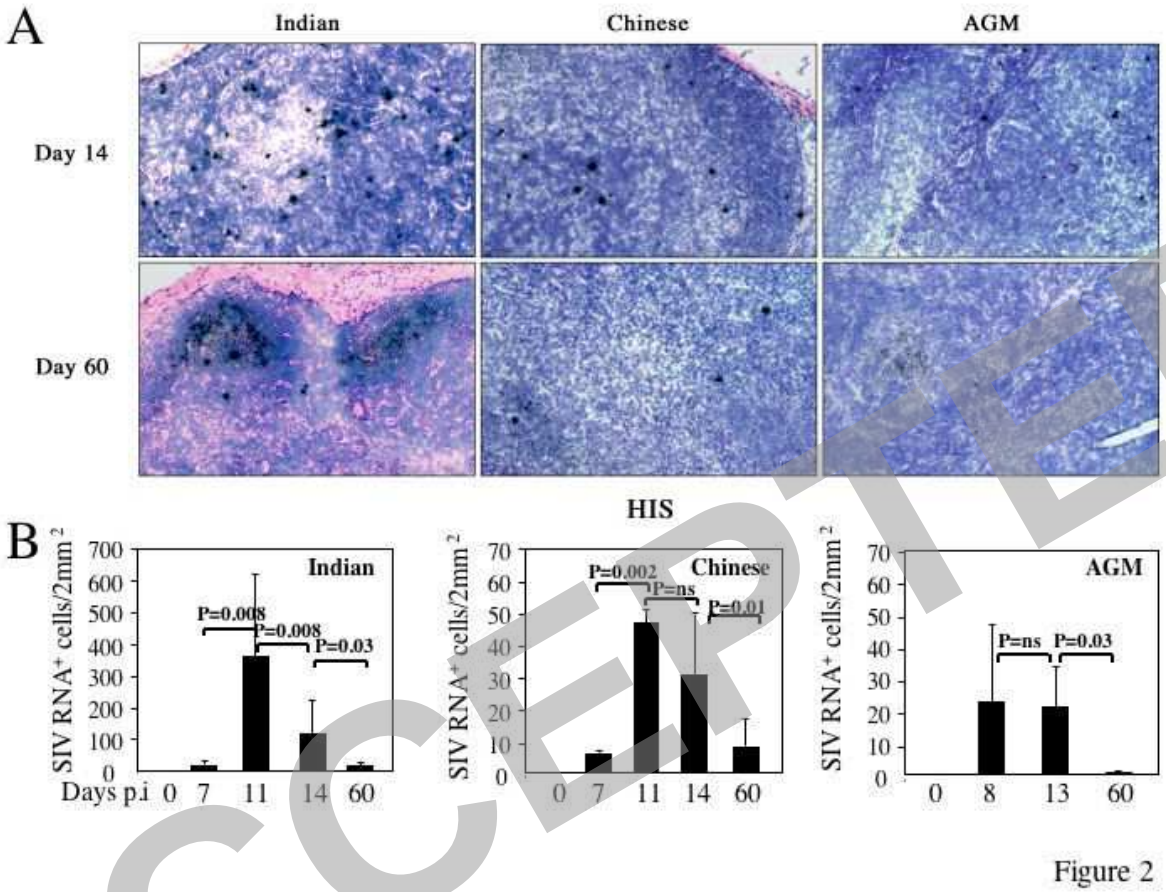

Figure 2

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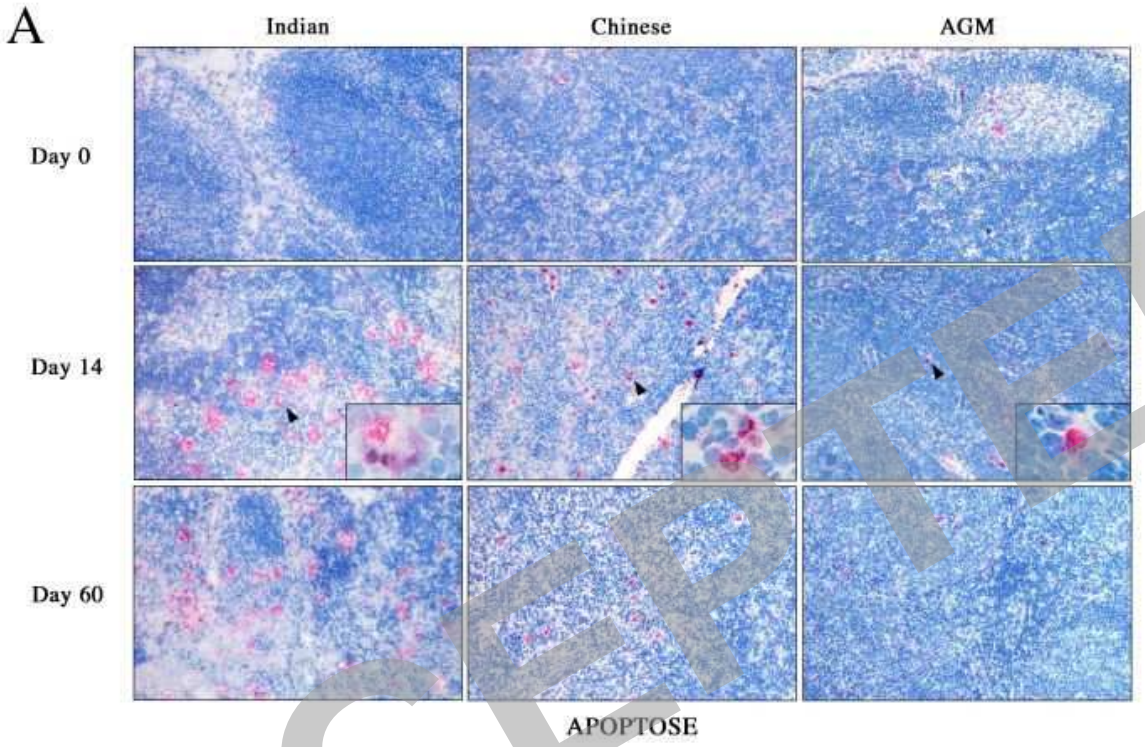

Figure 3

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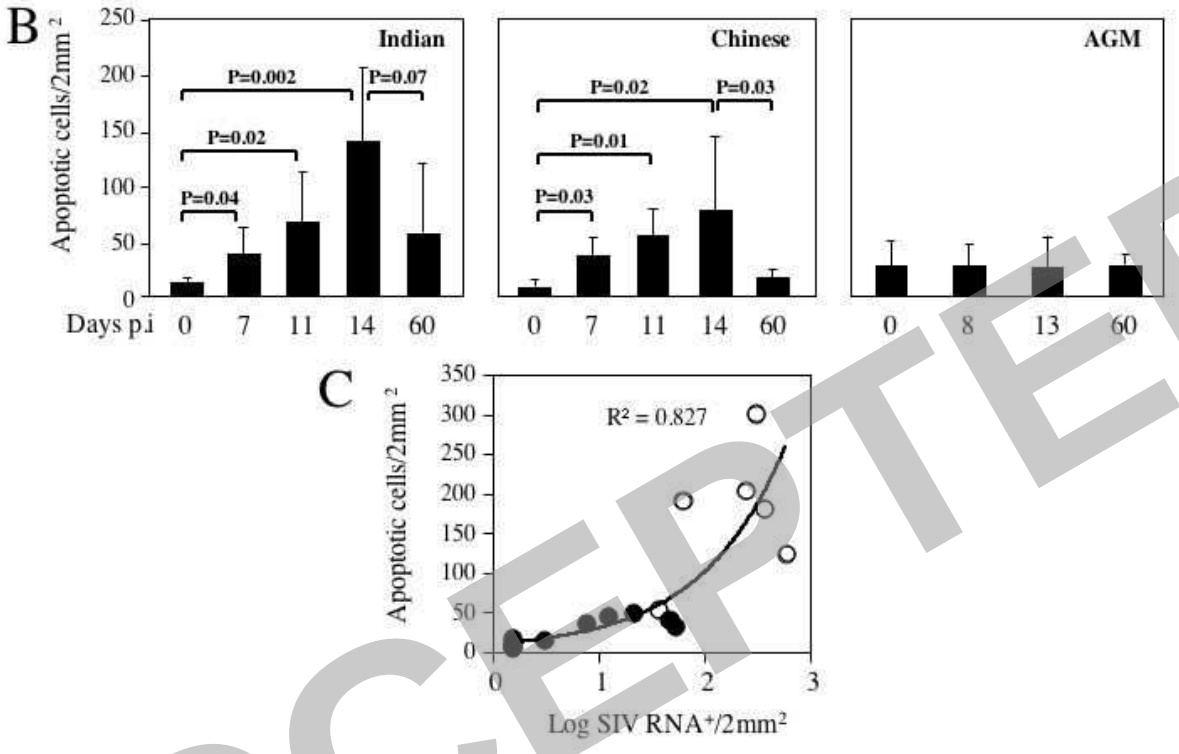

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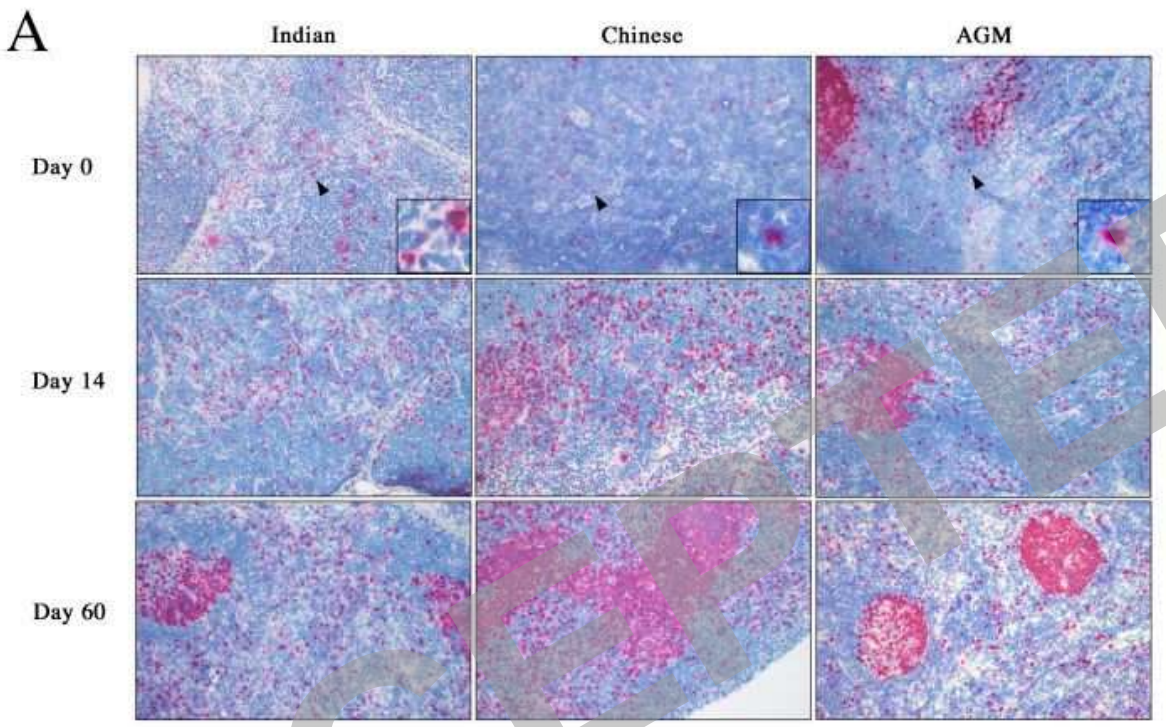

Figure 4

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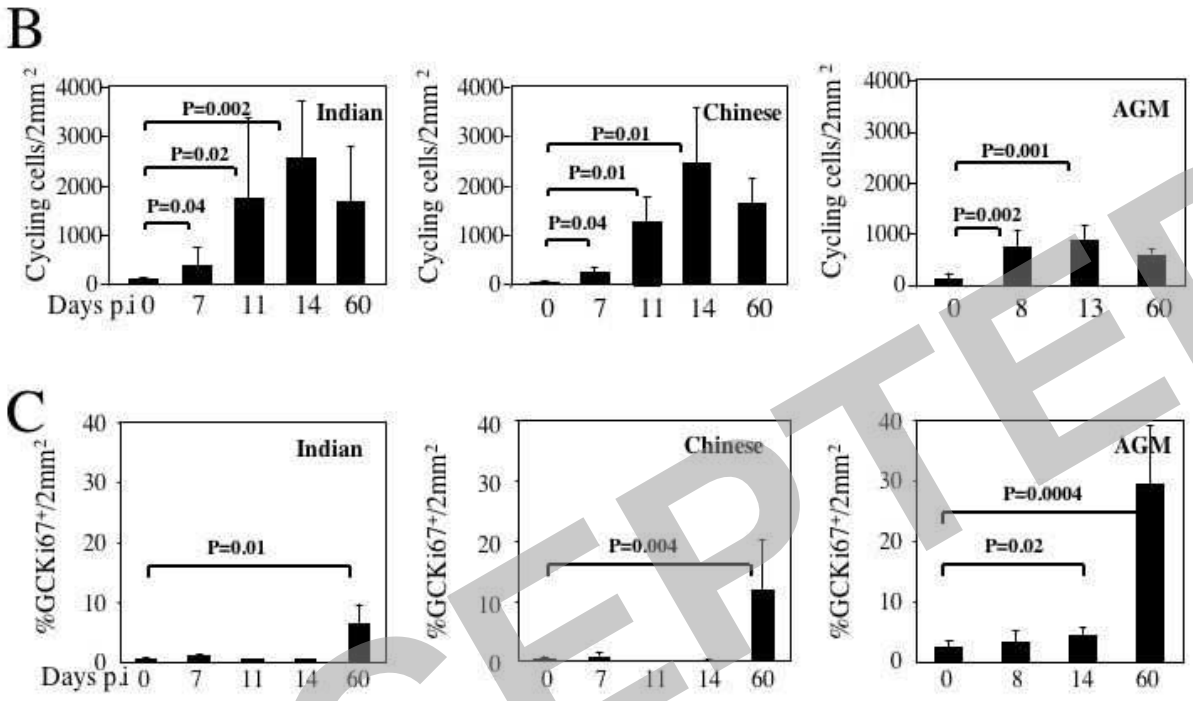

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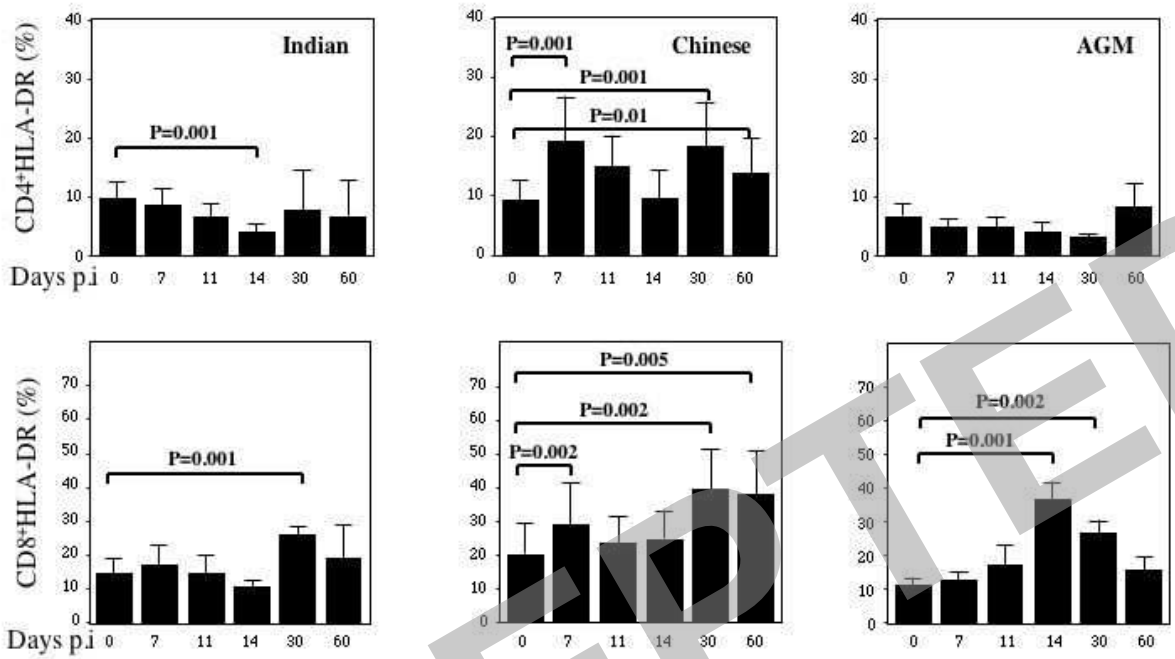

Figure 5

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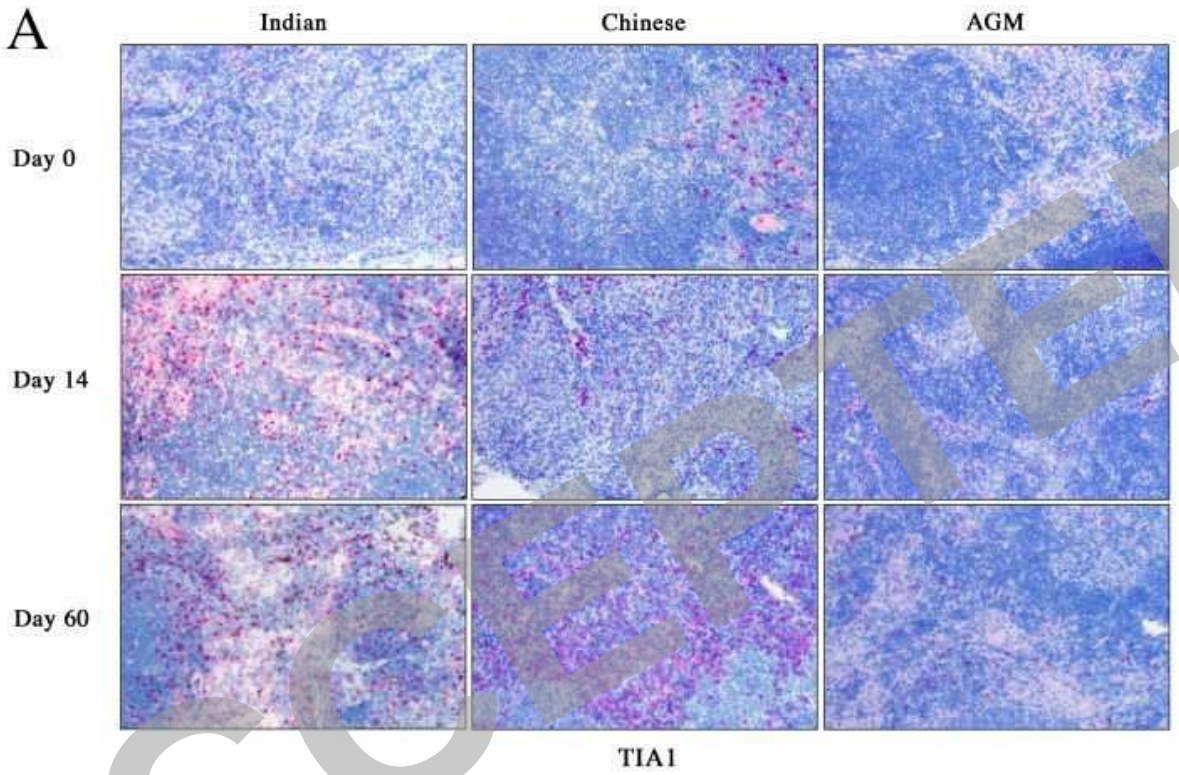

Figure 6

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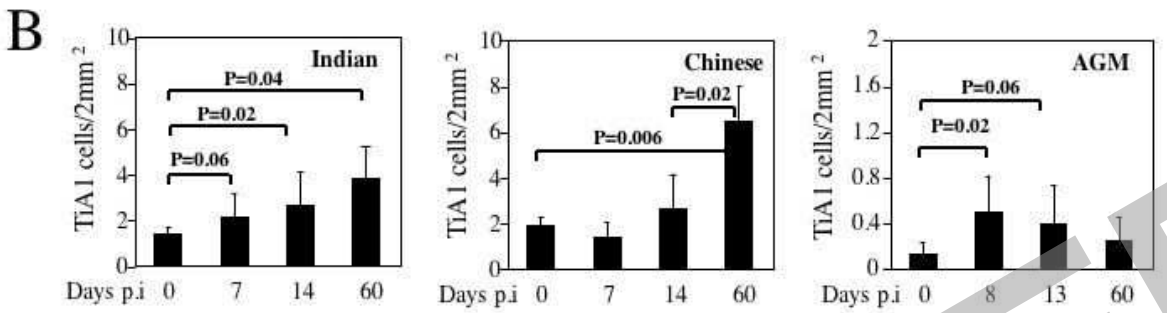

Figure 6

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