Acute-phase CD4+ T-cell proliferation and CD152 upregulation predict set-point virus replication in vaccinated simian–human immunodeficiency virus strain 89.6p-infected macaques

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INTRODUCTION

Human immunodeficiency virus (HIV) infection in humans and simian immunodeficiency virus (SIV) infection in rhesus macaques are typically characterized by a slow, progressive loss of CD4+ T cells leading to immune incompetence and the onset of opportunistic infections (Fauci, 1988; Lifson et al., 1988). However, already by the early stages of infection, gut-homing CCR5-expressing CD4 memory subsets are being depleted (Brenchley et al., 2004; Li et al., 2005; Mattapallil et al., 2005, 2004; Veazey et al., 1998, 2000) and CD4+ T-helper cell function is impaired (Clerici & Shearer, 1993; Koopman et al., 2001; Lane et al., 1985; McKay et al., 2003; Meyaard et al., 1996; Miedema et al., 1988; Shearer & Clerici, 1991). Recently, both events were found to be associated with the development of high virus load in SIV-infected macaques (Sun et al., 2007). Besides a possible direct immunosuppressive effect exerted by several HIV proteins (Cefai et al., 1992; Schindler et al., 2006; Westendorp et al., 1995), the formation of regulatory T cells, specifically the thymus-derived CD25hi/FOXP3+/CD152+ subset (Andersson et al., 2005; Estes et al., 2006; Hryniwicz et al., 2006; Kinter et al., 2004; Leng et al., 2002; Nilsson et al., 2006; Tsunemi et al., 2005), was found to be increased in HIV as well as in SIV infection, and was shown to contribute to suppression of HIV-specific immune responses (Kinter et al., 2004; Nilsson et al., 2006; Tsunemi et al., 2005).

On the other hand, HIV infection induces a state of chronic immune activation, as shown by increased expression of activation and proliferation markers and increased sensitivity to spontaneous and activation-mediated apoptosis induction (Carbone et al., 2000; Gougeon et al., 1993; Hazenberg et al., 2003; Meyaard et al., 1992; Miedema et al., 1988; Ribeiro et al., 2002). Chronic immune activation has been hypothesized
ultimately to exhaust the immune system, thus causing AIDS (Deeks et al., 2004; Hazenberg et al., 2003; Sousa et al., 2002). As the preferential depletion of memory cells and the induction of immune activation occur simultaneously, it is not possible to discern which of these two factors forms the main triggering event that leads to failure of virus control.

In contrast to HIV and SIV, the infection of macaques with pathogenic simian–human immunodeficiency virus strain 89.6p (SHIV89.6p) results in a rapid depletion of CD4+ T cells. However, several vaccination protocols have achieved preservation of CD4+ T cells (Amara et al., 2001; Barouch et al., 2000; Doria-Rose et al., 2003; Mooij et al., 2004; Shiver et al., 2002; Takeda et al., 2003; Voss et al., 2003), including the CD4+ memory subsets (Nishimura et al., 2004). A unique opportunity is thus provided to study immune activation and its relationship to the establishment of a set-point virus load in the absence of memory T-cell loss. In this study, a cohort of SHIV89.6p-infected animals, derived from a previous vaccine-evaluation study (Koopman et al., 2008), that showed various levels of control of virus replication was evaluated for activation-and proliferation-marker expression through the successive stages of acute and chronic infection. In addition, CD4+ memory/naive subset composition and expression of CD152, which can provide an inhibitory signal that counteracts cell activation, were studied. High peak-level expression of Ki-67 as well as CD152 in the acute phase of the infection, when virus replication was high in all animals, was found to correlate with a high set-point virus load in the chronic phase of the infection and was correlated inversely with HIV/SIV-specific gamma interferon (IFN-γ) responses observed immediately after challenge. Changes in the relative CD4+ memory/naive subset composition did not play a role.

**METHODS**

**Animals, immunizations and viral challenge.** Heparinized blood was obtained from captive-bred, mature (4–5-year-old), outbred, Chinese-origin rhesus macaques (Macaca mulatta). All animals were housed at the Biomedical Primate Research Center, Rijswijk, The Netherlands, according to international guidelines for non-human primate care and use. The animals had participated in a previous vaccine evaluation study (Koopman et al., 2008). In brief, five groups of six rhesus macaques were immunized at weeks 0, 8, 16 and 24 with either the pRix57 DNA vector encoding HIV-1W6.1D Env (gp120), SIV Nef and HIV-1 Tat, or a combination of HIV-1W6.1D Env, HIV-1 Nef and Tat and SIV Nef proteins formulated in the AS02A Adjuvant System. Animals received either DNA at all time points, DNA twice followed by protein twice. The control group received empty DNA vector plus AS02A at all time points. One animal in the DNA/protein vaccine group had to be euthanized for virus load in the chronic phase of the infection and was found to correlate with a high set-point virus load in the absence of memory T-cell loss. In this study, a cohort of SHIV89.6p-infected animals, derived from a previous vaccine-evaluation study (Koopman et al., 2008), that showed various levels of control of virus replication was evaluated for activation-and proliferation-marker expression through the successive stages of acute and chronic infection. In addition, CD4+ memory/naive subset composition and expression of CD152, which can provide an inhibitory signal that counteracts cell activation, were studied. High peak-level expression of Ki-67 as well as CD152 in the acute phase of the infection, when virus replication was high in all animals, was found to correlate with a high set-point virus load in the chronic phase of the infection and was correlated inversely with HIV/SIV-specific gamma interferon (IFN-γ) responses observed immediately after challenge. Changes in the relative CD4+ memory/naive subset composition did not play a role.

**Determination of virus load and cellular immunology assays.** A quantitative competitive RT-PCR was used to estimate the virus load in plasma as described previously (Ten Haaf et al., 1998). Induction of IFN-γ responses was measured using an ELISpot assay as described by Koopman et al. (2004). Separate peptide pools, consisting of 15mers with an 11 aa overlap, that covered HIV-1R7 gp120, HIV-1 Nef, SIVmac239 Nef (NIH) and SIVmac239 Gag (NIH) were used to measure antigen-specific immune responses against vaccine as well as challenge virus components.

**Antibodies.** The antibodies used in this study were directly coupled to Pacific Blue, fluorescein isothiocyanate (FITC), phycoerythrin (PE), peridinin–chlorophyll protein (PerCP), allophycocyanin (APC), phycoerythrin–cytochrome 7 (PE–Cy7) or allophycocyanin–cytochrome 7 (APC–Cy7). CD45RA was custom-biotinylated by Becton Dickinson. The following monoclonal antibodies (mAbs) were used: from Becton Dickinson/PharMingen, CD3Pacific Blue clone SP34.2, Ki-67FITC clone B56, CD29FITC clone L27, CD69FITC clone FN50, CD16PE clone B7.3.1, CD195PE (CCR5) clone 3A9, CD184PE (CXCR4) clone 12G5, CD107aPE clone HA43A, HLA-DRPerCP clone L243, CD3PerCP clone SP34.2, CD3APC clone SP34, CD152APC clone BN13, integrin B7APC clone Fib504, CD4APC–Cy7 clone SK3, CD8APC–Cy7clone SK1 and CD45RAbiotin clone 5H9; from R & D Systems, CCR7FITC clone FAB197F; from Miltenyi Biotec GmbH, CD25PE clone 4E3; and from AbD Serotec, CD62LIP clone FMC46.

**CD4+ T-cell counts and phenotyping.** Quantitative changes in peripheral blood mononuclear cell (PBMC) subsets after challenge were monitored by FACS analysis as described previously (Koopman et al., 2004). The following mAb combinations were used: (a) CD20FITC, CD16PE, HLA-DRPerCP, CD3APC, CD4PE–Cy7 and CD8APC–Cy7; (b) CD69FITC, CD29FITC, CD45RAbiotin, CD3APC, CD4PE–Cy7 and CD8APC–Cy7; (c) CCR7FITC, CD62PE, CD45RAbiotin, CD3APC, CD4APC–Cy7 and CD8APC–Cy7; (d) Ki-67FITC, CD10PE, CD3APC, CD4PE–Cy7, CD8APC–Cy7 and CD45RAbiotin; (e) CD3Pacific Blue, CCR7FITC, CD195PE, CD45RAbiotin, integrin B7APC, CD4APC–Cy7 and CD8APC–Cy7; (f) CD3Pacific Blue, CCR7FITC, CD184PE, CD45RAbiotin, integrin B7APC, CD4APC–Cy7 and CD8APC–Cy7.

For staining of surface markers, 100 μl heparin-treated blood was incubated with a mAb mix containing 2.5 μl of each mAb in 5 ml polystyrene round-bottomed tubes (Falcon 2058; Becton Dickinson) at room temperature for 15 min. After this incubation, cells were washed with 1 ml PBS and centrifuged for 5 min at 500 g. Subsequently, tubes b, c, e and f were incubated with PerCP-labelled streptavidin (Becton Dickinson) for 15 min at room temperature. Subsequently, 1.5 ml lysis solution (Becton Dickinson) was added to all tubes, followed by incubation at room temperature for 10 min and then centrifugation for 5 min at 500 g. The supernatant was aspirated and the cells were either resuspended in 5 ml PBS with 1–2% formaldehyde (tubes a, b, c, e and f) or incubated with a mixture of Ki-67FITC and CD152APC diluted in Becton Dickinson perm/wash solution (tube d) for 30 min at room temperature in the dark. After washing with perm/wash solution, 5 ml PBS with 1–2% formaldehyde was added and all samples were stored overnight at 4°C. Polystyrene Fluorospheres ( Beckman Coulter) were used to calculate absolute lymphocyte count. Flow cytometry was performed on a FACSaria machine using Diva software (Becton Dickinson). For each tube, 20,000 events in the lymphocyte gate were recorded.

**Statistical analysis.** Correlation between CD4+ T-cell count, CD195, CD184, HLA-DR, Ki-67 and CD152 expression on CD4+ T cells, HLA-DR and Ki-67 expression on CD8+ T cells and set-point virus load [at week 40 post-infection (p.i.)] or set-point CD4+ count (at week 40 p.i.) was calculated using a two-tailed Spearman correlation test. A two-tailed Spearman correlation test was also used to calculate the correlation between IFN-γ ELISPot responses and HLA-DR, Ki-67 and CD152 expression on CD4+ T cells.
RESULTS

CD4⁺ T-cell count and subset changes following SHIV89.6p challenge in a cohort of vaccinated and control animals

Previous studies in HIV-infected humans as well as in SIV-infected rhesus macaques have shown a concomitant loss of T-helper function, preferential loss of central memory T cells and increased expression of activation and proliferation markers, which were all correlated with high virus load or indicative of disease progression (Deeks et al., 2004; Hazenberg et al., 2003; Li et al., 2005; Mattapallil et al., 2005, 2004; Shearer & Clerici, 1991; Sopper et al., 2000; Sun et al., 2007). Here, we studied a cohort of SHIV89.6p-infected animals. Twenty-three of the animals used in this study had been immunized against HIV/SIV via single or combined DNA and protein administration, whilst six animals had received empty DNA vector/adjuvant only (see Methods). All animals became infected after challenge with SHIV89.6p (Fig. 1). Whilst all six control animals maintained detectable virus replication levels throughout the study, varying from $>10^5$ to as low as 500 copies ml⁻¹, the immunized animals showed some level of control, with undetectable virus load in 12 of 23 animals, low to intermediate virus replication in ten animals and relatively high virus replication of $>10^4$ copies ml⁻¹ in one animal (Fig. 1). The results are presented using this distinction, with vaccinated animals that develop undetectable virus load placed in one group and vaccinated animals that had a variable and detectable virus load placed in a second group. As shown in Fig. 1, almost all of the animals in the control group (top row) experienced a strong transient decrease in CD4⁺ T-cell count, which was most profound at 3 weeks p.i. However, CD4⁺ T cells then recovered almost to pre-infection levels in three animals, which also had the low virus load (Fig. 1). In two other animals, the level of CD4⁺ T cells remained somewhat reduced, whilst in one animal, which developed AIDS and had to be euthanized at week 20 p.i., CD4⁺ T cells remained almost undetectable. Similar transient decreases were seen in six of 23 immunized animals, who all largely recovered, except for one animal that also developed AIDS and was euthanized at week 29. During the course of the infection, both the CCR7⁺/CD45RA⁻ central memory CD4⁺ T-cell subset as well as the CCR7⁻/CD45RA⁻ effector memory CD4⁺ T-cell subset remained intact in almost all animals (Fig. 1), and only one of the immunized animals and two of the control animals experienced a relative decrease in the percentage of central memory CD4⁺ T cells. The CD4⁺ count at its low point at 3 weeks p.i., as well as at the end point at 40 weeks p.i., was correlated inversely with steady-state virus load (Fig. 2) with high statistical significance. The percentage of central memory CD4⁺ T cells in these SHIV89.6p-infected macaques did not correlate with virus load (Fig. 2), in contrast to what is seen in SIV-infected macaques.

To evaluate further the possible preferential depletion of co-receptor CD195 (CCR5)- or CD184 (CXCR4)-expressing cells or of cells expressing the integrin β7 marker, which is expressed preferentially on gut-homing cells, expression of these markers was studied at week 40 p.i. on CD4⁺ and CD8⁺ cells. As shown in Fig. 2, CD195- as well as CD184-expressing CD4⁺ T cells were clearly detectable and thus remained present in the infected animals. Moreover, expression of these markers did not correlate with steady-state virus load. Similar results were obtained for integrin β7 expression (not shown). Thus, in this cohort of animals, CD4⁺ memory as well as the CD195- and CD184-expressing subsets were relatively well maintained, despite a transient loss of CD4⁺ T cells in the acute phase of the infection.

CD4⁺ T-cell activation, proliferation and CD152 expression following SHIV89.6p challenge

In order to study T-cell activation and proliferation and induction of CD152, blood samples were stained for HLA-DR, CD69, Ki-67 and CD152, as a cell-activation inhibitory marker, during the course of the infection. As shown in Fig. 3(a), a strong increase in HLA-DR, Ki-67 and CD152 expression was seen on CD4⁺ T cells, which was most marked in the control group of non-vaccinated animals (Fig. 3a, top row). Peak-level expression of these markers was reached at 8, 4 and 4 weeks, respectively, after challenge. In contrast, expression of CD25 and CD69 on CD4⁺ T cells was relatively stable (Fig. 3a and not shown). In almost all animals, a CD25hi-expressing subset could be discriminated, which has been described to represent regulatory T cells (Kinter et al., 2004). Separate analysis revealed that 20–40% of the total CD25 population belonged to the CD25hi subset. However, during infection, the number of these cells was largely unaltered (Fig. 3a). Increases in Ki-67 expression were also observed in the CD8⁺ T-cell population, reaching a peak at 3 weeks p.i. (Fig. 3b). Expression of HLA-DR was more variable, whilst CD152 expression remained negative on CD8⁺ T cells. Although CD8⁺ T cells did express CD25, the percentage of positive cells was lower than in CD4⁺ T cells and did not change during infection (not shown).

Peak expression levels of Ki-67 as well as CD152 on CD4⁺ T cells, observed in the acute phase of the infection, were correlated positively with set-point virus load (Fig. 4) and correlated inversely with CD4⁺ count (not shown), whilst expression of HLA-DR on CD4⁺ T cells at this time point did not correlate with set-point virus load. In contrast to previously published human data (Hazenberg et al., 2003), there was no correlation between set-point virus load and levels of Ki-67 expression seen on CD4⁺ T cells immediately before challenge, indicating that the level of cell activation at the time of viral challenge had no predictive effect (not shown). Although Ki-67 was clearly upregulated on CD8⁺ T cells, this did not correlate with virus replication and neither did expression of HLA-DR (Fig. 4). As in the chronic phase of infection, the expression of HLA-DR, Ki-67 and CD152 still tended to be elevated in comparison with the pre-infection expression levels (Fig. 3). Statistical
Fig. 1. Virus load, CD4$^+$ count and CD4$^+$ central and effector memory subset composition in vaccinated and control animals challenged with SHIV$_{89.6p}$. Virus load (RNA equivalents (ml plasma)$^{-1}$), peripheral blood CD4$^+$ count (cells $\mu$L$^{-1}$) and the percentage of CD4$^+$ central memory T cells (T$_{cm}$) (CCR7$^+$/CD45RA$^-$) and CD4$^+$ effector memory T cells (T$_{em}$) (CCR7$^-$/CD45RA$^+$) are depicted against time after viral challenge for individual animals in the group of control animals (top row), the vaccinated animals that became virus-negative (middle row) and the vaccinated animals that stayed virus-positive (lowest row). The two animals that developed AIDS and had to be euthanized during the course of the study are indicated as dotted lines. VL, Virus load.
analysis was performed on the end-point (week 40) values of these markers. Week 40 HLA-DR and Ki-67 expression on CD4+ as well as on CD8+ T cells correlated positively with set-point virus load (Fig. 4). Expression of CD152 at this stage on CD4+ T cells did not correlate with virus load. As CD152-expressing cells could accumulate in the lymphoid tissues during the course of HIV infection, mesenteric lymph-node tissue was further analysed at 40 weeks p.i. (Andersson et al., 2005; Epple et al., 2006; Estes et al., 2006; Nilsson et al., 2006). Although in the lymph node 5–25% of CD4+ T cells were found to express CD152, there was no correlation with virus load (not shown), in stark contrast to what was seen at peak expression in the blood.

The typical pattern of early post-infection upregulation of activation, proliferation and CD152 marker expression lead us to plot these parameters against the previously reported early post-infection HIV/SIV-specific immune responses (Koopman et al., 2008). As demonstrated in Table 1, an inverse correlation between antigen-specific IFN-γ production, measured at 2, 8 or 12 weeks p.i., and peak levels of Ki-67 and CD152 expression was observed, whilst comparison with peak-level HLA-DR expression did not reach statistical significance. IFN-γ responses measured at later time points did not correlate with CD4+ T-cell Ki-67 or CD152 expression (Table 1). Thus, induction of immune activation seemed less pronounced in animals that were able to make good virus-specific IFN-γ responses early after challenge.

**DISCUSSION**

Despite the gradual progressive loss of CD4+ T cells and slow progression towards AIDS, early functional defects in CD4+ T-helper function, as well as an acute loss of gut-homing, CD195-expressing memory CD4+ T-cell populations, have been noted, both in HIV-infected humans and in SIV-infected rhesus macaques (Brenchley et al., 2004; Carbone et al., 2000; Hazenberg et al., 2003; Koopman et al., 2001; Li et al., 2005; Mattapallil et al., 2005; McKay et al., 2003; Ribeiro et al., 2002; Veazey et al., 1998, 2000). Recently, in a small cohort of SIV-infected macaques, the loss of both memory CD4+ T cells and cytokine-producing capacity was found to correlate with the development of a high set-point virus load (Sun et al., 2007). Here, we have described how, in a cohort of immunized/naïve, SHIV89.6p-infected rhesus macaques, with an unchanged naïve/memory CD4+ T-cell subset composition, the development of a high set-point virus load was associated strongly with the acute-phase CD4+ T-cell count, the proportion of dividing CD4+ T cells and CD152 upregulation.
Fig. 3. Activation, proliferation and CD152 marker expression dynamics in vaccinated and control animals challenged with SHIV89.6p. (a) The percentage of peripheral blood CD4+ T cells expressing HLA-DR, Ki-67, CD152 and CD25 in the group of control animals (top row), the vaccinated animals that became virus-negative (middle row) and the vaccinated animals that stayed virus-positive (lowest row) is shown. For CD25, two different subpopulations could be discriminated, i.e. with a high and intermediate expression. Depicted is the total CD25 expression (CD25hi plus CD25int, straight lines) with the CD25hi subpopulation (stippled lines) shown separately. (b) The percentage of peripheral blood CD8+ T cells expressing HLA-DR or Ki-67, measured over the 40 week course of the infection. VL, Virus load.
Chronic loss of CD4+ T cells is one of the strongest predictors for progression to AIDS. However, recent studies have identified pre-seroconversion low CD4+ counts and high levels of T-cell activation, as well as the post-infection proportion of dividing CD4+ and CD8+ T cells and levels of CD4+ T-cell activation, as additional, independent predictors of disease progression (Hazenberg et al., 2003). Our data in rhesus macaques infected with pathogenic SHIV seem to confirm some of these previous observations and, in addition, demonstrate that the increases in HLA-DR activation and Ki-67 proliferation marker expression are induced independently from changes in memory/naive CD4+ T-cell composition.

As evaluation of CD38, which is generally used for assessing cell activation in humans in addition to HLA-DR (Carbone et al., 2000; Deeks et al., 2004; Froebel et al., 2000; Hazenberg et al., 2003), is problematic in rhesus macaques, we chose to study CD69 and CD25 instead as additional markers for cell activation. However, expression of these markers was stable throughout the course of the infection. It should be noted that CD25 is expressed on activated as well as regulatory T cells, which can be discriminated as CD25hi cells (Kinter et al., 2004). However, further analysis showed that the percentage of CD25hi-expressing CD4+ T cells also did not change.

Increased expression of Ki-67, which discriminates dividing cells, has been described in HIV as well as SIV infection, both on CD4+ and CD8+ T cells, and was seen in the acute as well as the chronic phase of infection (Hazenberg et al., 2000, 2003; Sopper et al., 2000; Sun et al., 2007;
Role of acute-phase CD4+ activation in virus control

Table 1. Correlation between IFN-γ ELISPot responses and peak-level expression of activation, proliferation and regulatory T-cell markers on CD4+ T cells after SHIV89.6p challenge

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<tr>
<th>T-cell marker</th>
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<th>Week 12</th>
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<td>CD4+ HLA-DR (week 8)</td>
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<td>CD4 Ki-76 (week 4)</td>
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<tr>
<td>R</td>
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<td>-0.472</td>
<td>-0.518</td>
<td>NS</td>
<td>NS</td>
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<td>P</td>
<td>0.0003</td>
<td>0.01</td>
<td>0.004</td>
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<td>CD4+ CD152 (week 4)</td>
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<tr>
<td>R</td>
<td>-0.5</td>
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<td>-0.475</td>
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<td>P</td>
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Zaunders et al., 2005). Whilst induction of cell proliferation can be viewed as an indicator of preserved immune function (Sun et al., 2007), most reports instead describe a correlation with high virus load and disease progression (Hazenberg et al., 2003; Sopper et al., 2000). Furthermore, HLA-DR and Ki-67 are not increased in HIV-1-infected chimpanzees or SIV-infected sooty mangabeys, where disease development generally does not occur (Gougeon et al., 1997; Silvestri et al., 2005; Sumpter et al., 2007). Here, we have reported a similar upregulation of Ki-67 in SHIV89.6p-infected animals. Furthermore, Ki-67 peak expression on CD4+ T cells correlated positively with virus load and inversely with CD4 count. Of note is that Ki-67 expression on CD8+ T cells, although similarly increased, had neither an inverse nor positive correlation with virus load. The data therefore seem to indicate that, in the infection model studied here, the deleterious effects of cell activation outweigh the possible beneficial effects of antigen-triggered immune-activation induction. Pre-infection levels of Ki-67 expression on CD4+ T cells did not correlate with set-point virus load and, in contrast to recent observations in HIV-infected humans, did not seem to form an indicator for increased risk of development of AIDS (Hazenberg et al., 2003). However, the small number of animals involved may have prevented detection of more subtle associations.

Recently, CD25+/FOXP3+/CD152+ regulatory T cells were shown to be increased during HIV as well as during SIV infection and have been implicated in suppression of antiviral immune responses (Andersson et al., 2005; Estes et al., 2006; Hryniwicz et al., 2006; Montes et al., 2006; Nilsson et al., 2006). SHIV89.6p-infected animals showed a similar increase in CD152- but not in CD25hi-expressing CD4+ T cells in the acute phase of infection. As reported for HIV-infected humans (Leng et al., 2002; Tsunemi et al., 2005), CD152 peak expression level correlated inversely with CD4+ count and, in addition, correlated positively with virus load. However, no direct evidence for an immunosuppressive role of these cells is presented here. Indeed, the fact that there was no increase in CD25hi-expressing CD4+ T cells, as well as our observation that almost all of the CD152-expressing cells were also expressing Ki-67 (not shown), may indicate that, in the acute phase of the infection, expression of CD152 serves as an activation marker, and its correlation with virus load and CD4 count thus resembles the other activation markers. Furthermore, peak-level expression of Ki-67, HLA-DR and CD152 were mutually highly correlated (Ki-67 vs CD152, P<0.0001; HLA-DR vs Ki-67, P=0.0003; HLA-DR vs CD152, P=0.01; Spearman rank correlation test), supporting this supposition.

Accumulation of CD152-expressing cells in mesenteric lymph nodes as described in HIV and SIV infection (Andersson et al., 2005; Estes et al., 2006; Karlsson et al., 2007; Nilsson et al., 2006; Zaunders et al., 2006) was monitored in the SHIV89.6p-infected animals at the end of the study (week 40 p.i.). Although at this time point expression was low on peripheral blood T cells, high numbers of CD152-expressing CD4+ T cells were seen in the lymphoid tissues. However, these cells had low (20–30%) Ki-67 expression and thus seemed to be less activated (not shown). No correlation with set-point virus load was observed, which, together with the preservation of antiviral immune responses reported previously (Koopman et al., 2008), contradicts a role for regulatory T-cell-mediated immune suppression in this model.

As also noted in acute HIV and SIV infection (Fauci, 1988; Gruters et al., 1991; Horton et al., 2002; Sun et al., 2007), the SHIV89.6p-infected animals experienced a transient decrease in CD4+ T cells. Whilst this has previously been noted in SHIV89.6p-challenged immunized animals, it is unusual for the challenge control group. However, the challenge virus was clearly pathogenic, as many animals developed a high set-point plasma viraemia and two animals had to be euthanized during the study because of the development of AIDS. The fact that Chinese-origin rhesus macaques were used in this study may have led to a different pattern of CD4+ T-cell loss (Marcondes et al., 2006; Reimann et al., 2005) that better resembles the course
of events following HIV and SIV infection, but with the marked difference that gut-homing CD195-expressing cells were preserved. In comparison with SHIV89.6P-infected Indian rhesus macaques, these animals developed relatively high-level immune responses, which may have contributed to the preservation of CD4+ T cells (Koopman et al., 2008; Reimann et al., 2005). It must be noted that the control group in this study received empty DNA vector plus adjuvant and that an untreated control group was not included. However, in previous experiments, it was found that peak virus load and steady-state (week 40) virus load were not statistically significantly different in untreated control animals.

As reported previously (Koopman et al., 2008) and shown in Fig. 1, various levels of virus control were achieved in the cohort of animals studied here and these were correlated inversely with early post-challenge (week 2), anamnestic, virus-specific IFN-γ production, but not with responses measured at later time points or pre-infection. Importantly, this antigen-specific IFN-γ production, which was directed mainly against SIV Gag and Nef, was inversely correlated with peak-level Ki-67 and CD152 expression. In conclusion, the level of CD4 loss as well as CD4+ T-cell stimulation that is experienced in the acute phase of infection, which seem to be mutually correlated events, appear to determine the further development and eventual set point of virus replication. These changes were found to take place even though no selective loss of CD4+ memory T-cell subpopulations was seen and in the presence of preserved immune function (Koopman et al., 2008) and thus constitute an independent predictive factor. Finally, the inverse correlation with antigen-specific, early post-challenge IFN-γ responses suggests that immune protection may exert its strongest effect in the acute phase.

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