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Short Communication

Characteristics of Effective Immune Control of Simian/Human Immunodeficiency Virus in Pigtail Macaques

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ABSTRACT

Considerable evidence suggests both HIV-specific T cells and neutralizing antibodies (nAb) can, separately, assist control of viremia. T cell and nAb responses were studied in detail in three pigtail macaques protected from chronic simian/human immunodeficiency virus (SHIV) viremia by DNA prime/fowlpoxvirus boost vaccine regimens. Immunity was studied both after an initial intrarectal SHIV challenge, as well as during CD8 T cell depletion and a subsequent intravenous SHIV rechallenge. Remarkably, SHIV-specific CD4 and CD8 T cells were detectable in the absence of viremia following an initial SHIV challenge in one animal, subsequent to recovery from CD8 T cell depletion in all three animals, and following control of heterologous SHIV rechallenge in two animals. Neutralizing antibodies were also enhanced following CD8 depletion without recrudescence of viremia in all three animals. These observations, although in a small subset of animals, suggest the hypothesis that combinations of primed T cell immunity and neutralizing antibodies can maintain control of chronic primate lentiviral infections.

INTRODUCTION

UNDERSTANDING, AND INDUCING, EFFECTIVE IMMUNITY TO HIV remains a global priority. Induction of broad neutralizing antibody (nAb) to HIV by vaccines has not been achievable to date. Many current HIV-1 vaccine research efforts endeavor to primarily induce effective HIV-specific T cell immunity. CD8 and CD4 T-lymphocytes are important in controlling HIV-1 infection in humans and simian immunodeficiency virus/simian/human immunodeficiency virus (SIV/SHIV) infection in macaques, and generally correlate with protective immunity in macaque–SHVI studies.[1–4] Promising data have been obtained in nonhuman primate models, showing protection from AIDS, with large or complete reductions in SHIV viremia following the induction of SHIV-specific T cell immunity with DNA/poxvirus prime/boost vaccination regimens.[5,6] T cell responses also appear critical in the control of viremia in macaques and humans following transient early antiretroviral treatment.[7–11]

MATERIALS AND METHODS

We recently performed a challenge study of DNA and recombinant fowlpoxvirus (rFPV) vaccines expressing shared SHIV antigens in 30 pigtail macaques, 24 vaccinated with SHIV DNA and rFPV combinations, and 6 controls.[6] The DNA and rFPV vaccines expressed shared SIV_{mac239} *gag/pol* genes and expressed heterologous HIV-1 *env* genes. One or two 1 mg doses of the DNA were administered intramuscularly (IM), followed by 5×10^7 pfu of rFPV IM at 4 weekly intervals, followed by an intrarectal challenge 10 weeks later with a CXCR4-utilizing $SHIV_{mn229}$ stock. Overall, partial protection from SHIV viremia was observed, which correlated with T cell immunity. However, in contrast to rhesus macaque T cell-based vaccine studies utilizing $SHIV_{89,6P}$ challenges,[5,12,13] only a minority (three) of the 24 DNA/rFPV vaccinated pigtail monkeys controlled $SHIV_{mn229}$ viremia to undetectable levels within 11 weeks of initial challenge.

The control of SHIV in these three animals, however, offers the opportunity to probe the nature of the protective immune

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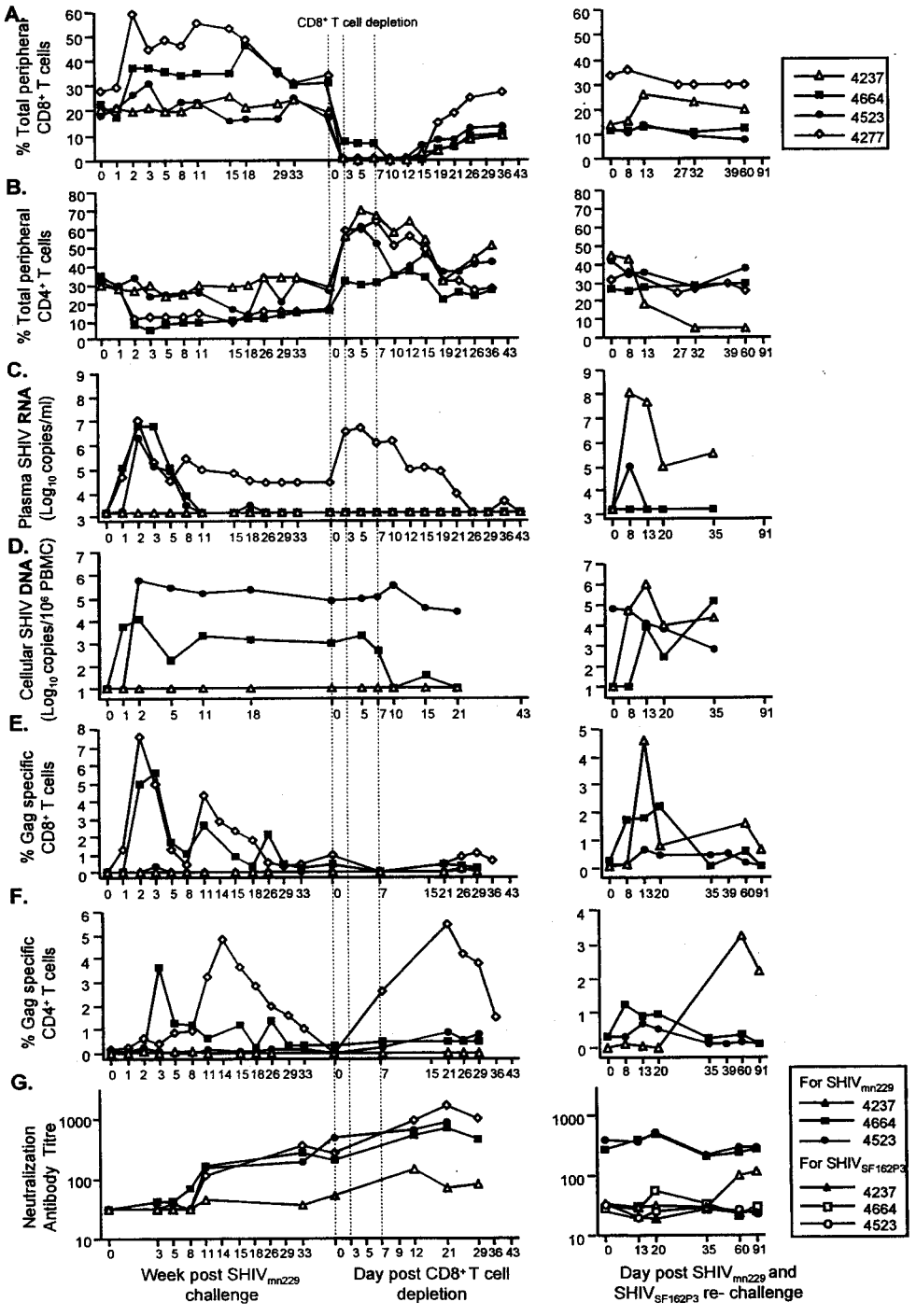

FIG. 1. Virologic and immunologic responses following SHIV challenge, CD8 depletion, and $SHIV_{mn229}/SHIV_{SF162P3}$ rechallenge. Each panel shows responses for 33 weeks following initial intrarectal $SHIV_{mn229}$ challenge (left panel); for up to 43 days following CD8 T cell depletion (three doses over 7 days, arrows) at week 36 following the initial challenge (left panel, right hand side); and for up to 91 days following a combined intravenous $SHIV_{mn229}/SHIV_{SF162P3}$ rechallenge at week 43 following the initial challenge (right panel). Symbols for the macaques studied in each panel are shown in (**A**) and (**G**). (**A**) Serial CD8 and (**B**) CD4 T cell counts in peripheral blood by flow cytometry. Levels are expressed as a percentage of total gated lymphocytes. (**C**) Plasma SHIV RNA by real-time PCR. (**D**) Total cellular SHIV DNA in PBMC by real-time PCR. Copies of cellular SHIV DNA were standardized to number of cells by real-time PCR for cellular CCR5. (**E**) SIV Gag-specific CD8 and (**F**) CD4 T cells expressing IFN-γ by intracellular cytokine staining of fresh whole blood and flow cytometric analysis. (**G**) nAb titers on TZM-bl cells using a luciferase reporter gene assay. After the combined $SHIV_{mn229}$ and $SHIV_{SF162P3}$ rechallenge (right panel), nAb were assayed to both viruses.

responses. To further characterize effective immunity to SHIV, we performed a detailed suite of virologic and immunologic analyses following a set of interventions (initial SHIV infection, CD8 T cell depletion, and combined homologous/heterologous SHIV rechallenge) to examine the nature of protective immunity in these apparently successfully immunized animals.

RESULTS

This study serially measured peripheral total CD4 and CD8 T cell levels by flow cytometry (Fig. 1A and B), plasma SHIV RNA, and cellular (PMBC) SHIV DNA by real-time PCR (Fig. 1C and D), SHIV-specific CD4 and CD8 T cell responses by intracellular interferon (IFN)-γ staining (Figs. 1E and F and 2), and nAb responses (Fig. 1G) in pigtail macaque blood samples using published assays.[6,14–18] We studied in detail 3 of 24 animals vaccinated with DNA/rFPV regimens that controlled plasma SHIV RNA levels to undetectable levels following $SHIV_{mn229}$ challenge. Virologic and immunologic responses were compared to another DNA/rFPV vaccinated pigtail macaque, animal 4277 (diamond symbols in Fig. 1), with stable chronic viremia (10^4–10^5 copies SHIV RNA/ml plasma, Fig. 1C) following intrarectal $SHIV_{mn229}$ challenge, which also underwent concurrent CD8 T cell depletion.

Initial intrarectal SHIV challenge

To first characterize immune responses during the control of the initial intrarectal $SHIV_{mn229}$ exposure, we studied cellular and humoral immunity in the three protected macaques after the $SHIV_{mn229}$ challenge. Two of the three "protected" animals (4664 and 4523) controlled $SHIV_{mn229}$ viremia following acute levels exceeding 10^6 copies SHIV RNA/ml (Fig. 1C, left panel). As expected, these animals had readily detectable levels of cellular SHIV DNA (Fig. 1D, left panel). Interestingly, although the initial levels of SHIV RNA were similar, there was a ~100-fold difference in levels of cellular SHIV DNA between these two animals. The animal with lower levels of cellular SHIV DNA (animal 4664) had higher levels of Gag-specific CD8 and CD4 T cell immunity by intracellular IFN-γ staining using a pool of overlapping SIV_{mac239} Gag 15mer peptides (similar to the comparator viremic animal 4277, Fig. 1E and F).

One animal, 4237, did not become detectably infected following the initial $SHIV_{mn229}$ challenge, with no cellular SHIV DNA or plasma SHIV RNA detected (Fig. 1C and D) and no seroconversion by Western blot (not shown). Gag-specific cellular immunity was not detected in peripheral blood samples from animal 4237 postchallenge (Figs. 1E and F and 2A). We have previously shown that Gag-specific T cell immunity was the predominant T cell response both primed by the Gag/Pol/Env-expressing DNA and rFPV vaccines and boosted following SHIV challenge in other animals.[6] Animal 4237 had neither Gag- or Pol-specific CD8 nor CD4 T cell immunity (<0.1% by ICS) detected postvaccination. Surprisingly, however, both SIV Pol-specific and HIV-1 Env-specific CD4 and CD8 T cell responses were detected in blood from animal 4237 following the SHIV challenge (Fig. 2A), suggesting that these responses were either primed at very low levels by the initial DNA/rFPV vaccination or boosted following a transient low

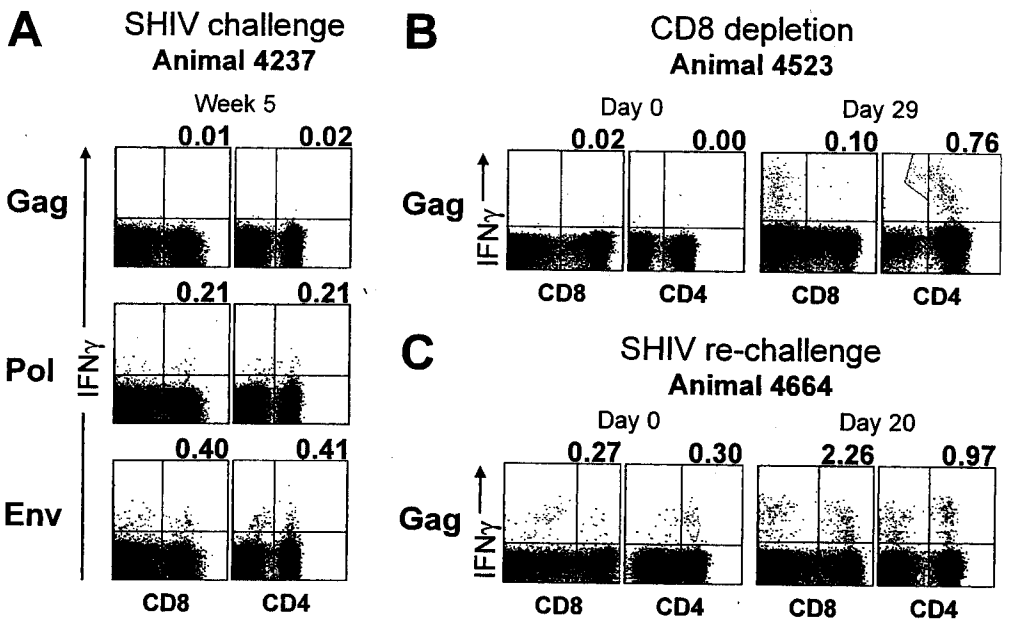

FIG. 2. T cell immune responses boosted in the absence of detectable viremia. Each panel shows the percentage of antigen-specific IFN-γ-expressing CD8 and CD4 T lymphocytes using sets of overlapping SHIV 15mer peptides. (**A**) SIV_{mac239} Gag-, SIV_{mac239} Pol-, and $HIV\text{-}1_{MN}$ Env-specific CD4 and CD8 T cell responses in animal 4237 5 weeks after an initial $SHIV_{mn229}$ challenge, which resulted in undetectable levels of SHIV RNA and DNA. (**B**) Gag-specific T cell responses in animal 4523 before and 29 days following CD8 T cell depletion, which did not result in detectable SHIV viremia. (**C**) Gag-specific T cell responses in animal 4664 before and 20 days following $SHIV_{mn229}$ and $SHIV_{SF162P3}$ rechallenge, which did not result in detectable SHIV viremia.

level infection not detected by SHIV RNA or DNA analyses. These results are consistent with reports of T cell immunity in HIV-1-exposed but uninfected humans.[19]

CD8 T cell depletion

CD8 T cell are widely recognized as an important immune response in controlling HIV/SIV infections. Transient CD8 T cell depletion of macaques by infusing an anti-CD8 mAb generally results in large increases in SIV/SHIV viremia.[20,21] To further probe the effective immunity in the three aviremic pigtail macaques, CD8 T cells were depleted for 10–12 days, 36 weeks following the initial SHIV challenge. We used the anti-CD8 mAb cM-T807 (generously provided by Keith Reimann, Harvard Medical Center, Boston, obtained from the National Cell Culture Center) administered subcutaneously (10 mg/kg) on day 0, and intravenously (5 mg/kg) on days 3 and 7 (Fig. 1A).[7,21] In the comparator viremic animal (4277), a transient ~100-fold increase in SHIV viremia ensued as expected,[20,21] but in the three aviremic animals, plasma SHIV RNA remained undetectable (Fig. 1C).

nAb responses are generally thought to be responsible for the complete absence in viremia ("sterilizing immunity") following SIV or SHIV exposure.[22,23] nAb responses were therefore assayed to the $SHIV_{mn229}$ challenge stock using a well-validated luciferase reporter gene assay.[6,17] The two animals with detectable acute viremia (4664 and 4523) gradually developed nAb to $SHIV_{mn229}$ 8 weeks after the initial infection despite undetectable levels of plasma viremia, but no significant nAbs were detected in animal 4237 prior to CD8 depletion (Fig. 1G, left panel). nAb titers to $SHIV_{mn229}$ were relatively stable from 26 weeks preceding CD8 depletion, however, within 2–3 weeks of CD8 depletion there was a mean 4.1-fold increase in nAb within all animals. This brisk rise in nAb levels suggests a role for nAb responses in maintaining control of viremia in the absence of CD8 T cells.

We also hypothesized that T cell immunity generated after vaccination or the initial challenge could be reactivated by small increases in virus levels following CD8 T cell depletion. Both animals 4523 and 4664 had Gag-specific T cell immunity detected following the initial SHIV challenge (Fig. 1C and D) and animal 4664 had high levels of Gag-specific CD8 T cells (0.26%) and CD4 T cells (0.18%) detected following vaccination. We therefore serially analyzed SIV-specific CD4 and CD8 T cells following CD8 T cell depletion. A significant increase in Gag-specific CD4 and CD8 T cell immunity evolved as CD8 T cells recovered on days 21–29 postdepletion, particularly in animal 4523 and, to a lesser extent, animals 4237 and 4664, despite the lack of detectable viremia (Figs. 1E and F and 2B).

Unexpectedly, the comparator animal 4277 reduced plasma SHIV RNA levels to undetectable levels (10- to 100-fold lower than the stable chronic levels predepletion) when CD8 T cells recovered postdepletion (Fig. 1C). This was associated with large expansions of both Gag-specific CD4 T cells and nAb responses. Further, animal 4664 had a sustained 100-fold reduction in cellular SHIV DNA following CD8 depletion (Fig. 1D). These observations require confirmation but suggest the hypothesis that even very low levels of recrudescent virus following CD8 depletion can induce or enhance effective non-CD8 T cell immune responses.

Heterologous SHIV rechallenge

To further assess the durability of protective immunity in these three animals, all three animals were then rechallenged intravenously simultaneously with 3×10^4 $TCID_{50}$ of the same stock of CXCR4-utilizing $SHIV_{mn229}$ used during the initial challenge, as well as 3×10^3 $TCID_{50}$, a heterologous CCR5-utilizing $SHIV_{SF162P3}$ stock.[24,25] This intravenous challenge occurred 44 weeks after the initial mucosal challenge and used similar amounts of virus that have successfully infected over 80 pigtail macaques via mucosal (rectal or vaginal) routes[6,16] (Kent *et al.*, submitted for publication). Since mucosal challenges are typically 100 to 1000-fold less efficient than intravenous challenges,[26] this intravenous exposure represented a high dose rechallenge.

Animal 4237 became infected following the intravenous rechallenge, with high levels of SHIV RNA and DNA and significant depletion of CD4 T cells (Fig. 1A–D, right panels). However, unlike naive $SHIV_{mn229}$-infected controls, CD4 T cell depletion was incomplete[6,16] suggesting some immunity. Indeed, brisk increases in Gag-specific CD8 and CD4 T cells ensued in animal 4237, later followed by an increase in $SHIV_{mn229}$-specific nAb (Fig. 1E–G), which are not observed in naive animals.

Animal 4664 had no detectable plasma SHIV RNA following this rechallenge, and in animal 4523 SHIV RNA was only detected once—8 days following rechallenge. Both these animals had large elevations in Gag-specific CD8 and CD4 T cells for several weeks after the combined homologous/heterologous rechallenge (Figs. 1E and F and 2C), likely reflecting expansion of T cell immunity primed by the vaccination or initial SHIV challenge. Although nAb titers were high for $SHIV_{mn229}$ prior to the rechallenge, titers remained unchanged, and there

TABLE 1. VIRUS SEQUENCE IN PBMC DNA FOLLOWING $SHIV_{mn229}$ AND $SHIV_{SF162P3}$ RECHALLENGE

Virus or macaque ID	*Days postrechallenge*	*Envelope sequence (amino acids 305–364)*
$SHIV_{mn229}$	Stock	SVSQRTGRAFVTTGKIGNMRQAHCNISRAKWNNNLKQIASKLREQYGNNRTIIFKQS
$SHIV_{SF162P3}$	Stock	...Y...K..YA..D.I.DI.....GE....T....VT..QA.FE.K..V....
4237	13	.....
4664	13	.....
4523	13	.....

were no detectable nAbs to the heterologous $SHIV_{SF162P3}$ stock (Fig. 1G).

To determine which of the two SHIV strains used in the rechallenge resulted in infection, HIV-1 Env sequences were amplified, cloned, and sequenced from PBMC DNA using conserved Env primers ENV1151–1134: TCTGGGTCCCCTCCTGAG and ENV931–950: AGACCYAACAAYAATACAAG with standard methodology.[27] In all three animals, the envelope fragment sequenced following the combined rechallenge was identical to the $SHIV_{mn229}$ stock (Table 1).

DISCUSSION

Taken together, this series of interventional analyses suggests that in the small number of effectively vaccinated macaques studied (1) control of viremia during CD8 depletion can occur concomitantly with SHIV-specific CD4 T cells and nAb responses and (2) heterologous rechallenge can be controlled coincident with increases in combinations of T cell and nAb immunity. Although anecdotal, our studies are consistent with observations on the roles of passive transfer of antibodies,[23] HIV-specific CD4 T cells following treatment of acute HIV-1 infection,[11] and induction of T cell immunity in HIV-1-exposed but uninfected humans.[19] Although these studies were limited by the small subset of three animals protected from initial SHIV challenge by DNA and rFPV vaccines, if confirmed in larger studies the results serve to highlight the potential breadth of acquired immune responses required to efficiently maintain long-term control of primate lentivirus infection.

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