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Vaccine Protection Against Functional CTL Abnormalities in Simian Human Immunodeficiency Virus-Infected Rhesus Monkeys¹

Paul F. McKay, Jörn E. Schmitz, Dan H. Barouch, Marcelo J. Kuroda, Michelle A. Lifton, Christine E. Nickerson, Darci A. Gorgone, and Norman L. Letvin²

Accumulating evidence suggests that HIV-specific CD8⁺ CTL are dysfunctional in HIV-infected individuals with progressive clinical disease. In the present studies, cytokine production by virus-specific CTL was assessed in the rhesus monkey model for AIDS to determine its contribution to the functional impairment of CTL. CTL from monkeys infected with nonpathogenic isolates of simian and simian-human immunodeficiency virus expressed high levels of IFN- γ , TNF- α , and IL-2 after in vitro exposure to a nonspecific mitogen or the optimal peptide representing a dominant virus-specific CTL epitope. However, similarly performed studies assessing these capabilities in CTL from monkeys infected with pathogenic immunodeficiency virus isolates demonstrated a significant dysfunction in the ability of the CTL to produce IL-2 and TNF- α . Importantly, CTL from vaccinated monkeys that effectively controlled the replication of a highly pathogenic simian-human immunodeficiency virus isolate following challenge demonstrated a preserved capacity to produce these cytokines. These experiments suggest that defects in cytokine production may contribute to CTL dysfunction in chronic HIV or SIV infection. Moreover, an AIDS vaccine that confers protection against clinical disease evolution in this experimental model also preserves the functional capacity of these CTL to produce both IL-2 and TNF- α . *The Journal of Immunology*, 2002, 168: 332–337.

While virus-specific CTL play a role in containing HIV-1 spread in infected individuals, accumulating evidence suggests that these effector lymphocytes may not be fully functional (1–5). The impaired functional ability of these virus-specific CTL was initially suggested by the observation that HIV-specific CTL show a progressive reduction in lytic activity as clinical disease progresses (6). This functional CTL impairment has been ascribed to a lack of CD4⁺ T cell help, HIV-1-induced changes in virus-specific CD8⁺ T cells, or a combination of these factors (7–10).

It is now possible to analyze with remarkable precision the phenotypic characteristics and functional capabilities of virus-specific CTL using MHC class I/peptide tetramers (11, 12). Studies employing this technology have detailed a variety of abnormalities in HIV-1-specific CTL that are associated with a loss of cytotoxic function (1, 13). These include abnormalities in the production of mediators of cytotoxicity as well as the expression of cell signaling molecules. The potential contribution of abnormal cytokine production to these defects, however, is still unclear.

Antiviral effects of selected cytokines have been well described. Both IFN- γ and TNF- α mediate potent antiviral responses (14, 15). In fact, the ability of CTL to secrete these cytokines is ac-

cepted as an indicator of their cytotoxic fitness. Whether CD8⁺ CTL in HIV-1-infected individuals have defects in cytokine secretion remains an unresolved issue.

We have utilized SIV-infected and simian-human immunodeficiency virus (SHIV)³-infected rhesus monkeys to investigate the biology of virus-specific CTL in the clinical setting of AIDS. SIV/SHIV-infected rhesus monkeys that express the MHC class I allele *Mamu-A*01* develop a dominant Gag-specific CTL response focused on the p11C epitope (16). Fluorochrome-labeled tetrameric Mamu-A*01/p11C complexes allow the analysis of these epitope-specific CD8⁺ CTL by flow cytometric methods (11, 17). This technique has recently allowed a precise characterization of the role of CTL in early AIDS virus clearance, the anatomic compartmentalization of CTL populations, and the V β gene repertoire utilized by AIDS virus-specific CTL (18–20).

The present studies were initiated to assess the functional capacity of CTL in SIV/SHIV-infected rhesus monkeys. In these experiments, Gag p11C tetramer-binding CD8⁺ T lymphocytes from infected *Mamu-A*01*⁺ monkeys were assessed for their ability to secrete IFN- γ , TNF- α , and IL-2. These studies show that virus-specific CD8⁺ T lymphocytes in animals with progressive disease exhibit a reduced capacity to secrete both IL-2 and TNF- α , and this loss is associated with a high viral load and low CD4⁺ T lymphocyte numbers. Interestingly, these studies also showed that vaccine protection against SHIV-induced clinical disease in this model was associated with preserved functional CTL capacity.

Materials and Methods

Animals and viruses

Heparinized blood samples were obtained from rhesus monkeys (*Macaca mulatta*). All animals were maintained in accordance with the guidelines of the Committee on Animals for the Harvard Medical School and the *Guide*

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³ Abbreviation used in this paper: SHIV, simian-human immunodeficiency virus.

for the Care and Use of Laboratory Animals (21). Viruses used in this study were the nonpathogenic SHIV-89.6, the J5 strain of SIVmac251 with low pathogenicity, and the highly pathogenic SIVsmE660 and SHIV-89.6P strains. SHIV-89.6 and SHIV-89.6P are chimeric viruses consisting of the SIVmac239 backbone and the HIV-1 89.6 envelope gene, which was cloned from a primary patient R5/X4 dual-tropic HIV-1 isolate (22).

CD4 counts and viral loads

Peripheral blood CD4⁺ T lymphocyte counts were determined by multiplying the total lymphocyte count by the percentage of CD3⁺ CD4⁺ T cells assessed by flow cytometry. Plasmid viral RNA levels were measured by a real-time RT-PCR amplification assay with a detection limit of 400–500 copies/ml, as described (23), using *gag* primers and probes (24).

Selection of Mamu-A*01⁺ rhesus monkeys

Rhesus monkeys were screened for the presence of the *Mamu-A*01* allele by a PCR-based technique, as previously described (25). EDTA-anticoagulated whole blood from rhesus monkeys was subjected to Ficoll diatrizoate density gradient centrifugation to isolate leukocytes, and the washed cell pellets were resuspended in 200 μ l PBS. DNA extraction was then conducted with a QIAmp blood kit (Qiagen, Chatsworth, CA). PCR was performed on 200–500 μ g extracted DNA with allele-specific primers in a 50 μ l reaction mixture consisting of 60 mM Tris (pH 8.5), 2 mM MgCl₂, 15 mM ammonium sulfate, 2 mM deoxynucleoside triphosphates (0.5 mM each), and 5 μ l *Taq* polymerase. Primers A*01/F (5'-GAC AGC GAC GCC GCG AGC CAA-3') and A*01/R (5'-GCT GCA GCG TCT CCT TCC CC-3') were used at final concentrations of 800 nM each. Two additional primers specific for a conserved MHC class II sequence (based on the rhesus monkey homologue of HLA-DRB3) were included in the reaction as an internal positive control. Primers 5' MDRB (5'-GCC TCG AGT GTC CCC CCA GCA CGT TTC-3') and 3' MDRB (5'-GCA AGC TTT CAC CTC GCC GCT G-3') were used at final concentrations of 680 nM each. PCR was conducted with a GeneAmp System 9600 thermocycler (PerkinElmer, Norwalk, CT). Samples were denatured at 96°C for 2 min, followed by 5 cycles of 25 s at 96°C and 60 s at 72°C; 21 cycles of 25 s at 96°C, 50 s at 67°C, and 45 s at 72°C; and 4 cycles of 25 s at 96°C, 60 s at 55°C, and 80 s at 72°C. The PCR products were analyzed by 1% agarose gel electrophoresis. Ten microliters of each PCR reaction mixture were loaded per lane.

Potential *Mamu-A*01*-positive animals were identified by the presence of two bands, a 685- and a 260-bp amplified product. DNA sequence analysis was then performed on all potential positive samples to confirm nucleotide sequence identity with the published *Mamu-A*01* prototype sequence (16). Before being sequenced, the amplified DNA was treated with 1 U per reaction of shrimp alkaline phosphatase and 10 U exonuclease I for 15 min at 37°C, followed by 15 min at 80°C. The sequencing templates were then purified with a QIAquick PCR purification kit (Qiagen). For each template, 70 ng DNA was used for DNA sequencing together with 5 pmol primer. Four PCR primers were used for sequencing: A*01/F and A*01/R, whose sequences are shown above, and B/1⁺ (5'-CTG CGC GGC TAC TAC AAC CA-3') and G/1⁺ (5'-ATG TAA TCC TTG CCG TCG TA-3'). Sequencing was conducted at a central core sequencing facility on an ABI-373 stretch DNA-sequencing machine, using ABI AmpliTaq FS dye terminator chemistry (PerkinElmer). All animals used in this study were genotypically *Mamu-A*01* positive based on the above screening, and were also *Mamu-A*01* positive as determined in a functional CTL assay.

Plasmid DNA vaccination protocol

The detailed vaccination protocol has been previously described (26, 27). Briefly, double-CsCl-banded maxipreparations of HIV-1 89.6P *env* (KB9) and SIVmac239 *gag* plasmid DNA were injected into both quadriceps muscles of rhesus monkeys. Animals treated with IL-2/Ig protein were inoculated twice daily with 0.5 mg/day for 14 days after DNA vaccine administration. Other animals received 5 mg IL-2/Ig plasmid on day 2 after DNA vaccination. The vaccination regimen consisted of inoculation at week 0, followed by boost immunizations at wk 4, 8, and 40. IL-2 treatments were included only at wk 0 and 4. Animals were challenged at wk 46 with 100 monkey infectious dose 50% (MID₅₀) SHIV-89.6P by the i.v. route.

*Mamu-A*01*/peptide tetramer complex formation and staining of PBMCs

*Mamu-A*01*/p11C tetramer complexes were prepared as previously described (11, 17). PE-labeled streptavidin (Prozyme, San Leandro, CA) was mixed stepwise with biotinylated *Mamu-A*01*/p11C peptide complexes at a molar ratio of 1:4 to produce the tetrameric complexes. All Abs used in

this study were directly coupled to FITC, PE-Texas Red (ECD), or allophycocyanin. The following mAbs were used: anti-CD3 FITC (SP34; BD PharMingen, San Diego, CA), anti-CD8 ECD (7PT; Beckman Coulter, Fullerton, CA), anti-IFN- γ allophycocyanin (B27; BD PharMingen), anti-TNF- α allophycocyanin (Mab11; BD PharMingen), and anti-IL-2 allophycocyanin (MQ1-17H12; BD PharMingen).

PBMC stimulation and intracellular cytokine staining

Unfractionated fresh PBMCs were cultured at 37°C in a 5% CO₂ environment for 6 h in the presence of RPMI medium alone (unstimulated) or RPMI medium containing 5 μ g/ml p11C (CTPYDINQM) minimal peptide (peptide stimulated) or 100 ng/ml PMA + 1 μ g/ml ionomycin (PMA + Iono stimulated). All cultures contained brefeldin A (10 μ g/ml, GolgiPlug; BD PharMingen) to disrupt Golgi apparatus transport, thereby causing the accumulation of intracellular cytokines, as well as 2 μ g/ml anti-CD28 and anti-CD49d. The cultured cells were stained with the *Mamu-A*01*/p11C tetramer and mAbs specific for cell surface molecules before RBC lysis and fixation (Immunoprep reagent system and Q-prep workstation; Beckman Coulter). The PBMCs were washed once with PBS/2% FCS, then permeabilized with Cell Fix/Perm solution (BD PharMingen), according to the permeabilization protocol. Cells were washed twice with 2.5 vol 1 \times Perm/Wash buffer (BD PharMingen) and then stained with 1 μ g anti-cytokine mAb/10⁶ cells. Anti-cytokine mAbs were titrated for optimal staining in preliminary experiments. Cells were washed twice with 2 ml 1 \times Perm/Wash buffer and once with PBS, and then fixed in 1.5% formaldehyde/PBS. Samples were analyzed on the FACSCalibur instrument using CellQuest software. Data analysis was performed using CellQuest software and presented in Microsoft PowerPoint 98 (Microsoft, Redmond, WA).

Statistical analyses

The mean cytokine expression percentages of each group were compared with two-sided Wilcoxon rank sum tests with Bonferroni adjustments of *p* values for the two major comparisons. A *p* value of <0.05 was considered significant.

Results

Rhesus monkey CD8⁺ CTL staining for intracellular cytokine expression

mAbs specific for human cytokines were assessed to determine whether they were suitable reagents for intracellular staining procedures to detect rhesus monkey cytokines. Selected mAbs specific for IFN- γ , TNF- α , and IL-2 stained permeabilized human and monkey PBMCs with equal intensity (Fig. 1), and were therefore used in all subsequent experiments.

Studies were then initiated to evaluate the expression of these cytokines by rhesus monkey CD8⁺ CTL specific for SIVmac Gag. PBMCs from an SIVmac-infected *Mamu-A*01*⁺ rhesus monkey were stimulated in vitro, stained with mAbs specific for IFN- γ , TNF- α , and IL-2, and evaluated by flow cytometric analysis, gating on p11C tetramer⁺CD3⁺CD8 $\alpha\beta$ ⁺ lymphocytes. These cells were cultured with a Golgi apparatus inhibitor so as to prevent cytokine secretion and allow the accumulation of cytokine protein in their disrupted Golgi network. Cells that received no in vitro stimulus demonstrated little accumulation of cytokines. The positive control PMA and ionomycin stimulation induced high levels of cytokine accumulation in these gated p11C tetramer⁺CD3⁺CD8 $\alpha\beta$ ⁺ T lymphocytes (Fig. 2). In cells stimulated with the SIV Gag p11C peptide, significant cytokine accumulation was seen in the Golgi network of the gated CTL. These observations suggested that this approach could be utilized to assess the potential for cytokine production by rhesus monkey CTL.

Cytokine expression by Gag epitope-specific CD8⁺ T cells in monkeys infected with nonpathogenic or pathogenically attenuated SIV and SHIV isolates

We first sought to assess the potential for cytokine production by CTL in a group of clinically healthy *Mamu-A*01*⁺ monkeys infected with the nonpathogenic SHIV-89.6 or an SIVmac251(J5) isolate with a relatively attenuated pathogenic potential. These

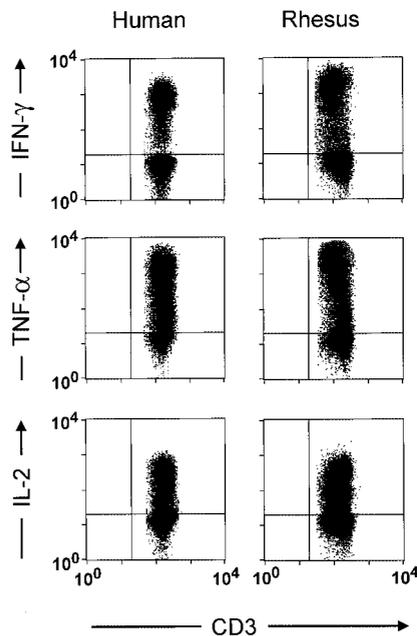


FIGURE 1. Equivalency of staining intensity with fluorochrome-labeled anti-cytokine mAbs in permeabilized human and rhesus monkey PBMCs. IFN- γ , TNF- α , and IL-2 staining in PMA + ionomycin-stimulated CD3⁺ T lymphocytes.

monkeys had been infected for more than 2 years, had maintained low viral loads and relatively normal CD4⁺ T cell counts, and showed no clinical signs of disease progression (Table I). PBMCs from all of these animals contained detectable populations of p11C tetramer⁺CD8 $\alpha\beta$ ⁺ T cells. Upon stimulation, these tetramer⁺CD8⁺ T cells expressed high levels of IFN- γ and TNF- α , and moderate levels of IL-2 (Fig. 3A). Specific Gag epitope peptide stimulation

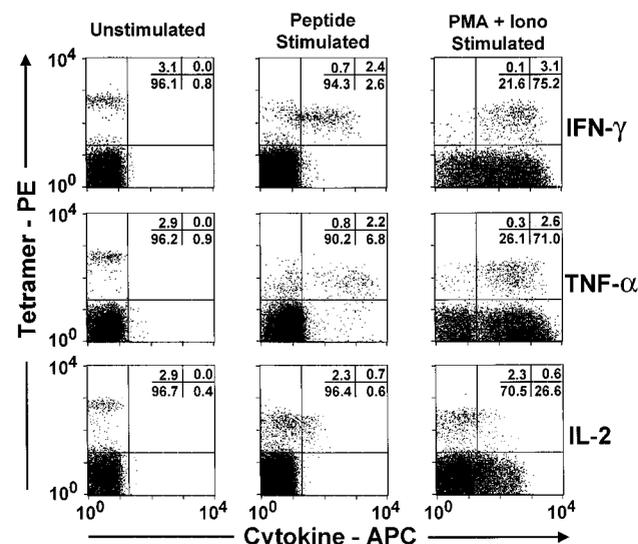


FIGURE 2. Typical cytokine-staining profiles in permeabilized rhesus monkey CD3⁺CD8⁺ and CD3⁺CD8⁺p11C tetramer⁺ T lymphocytes. *Top panels*, IFN- γ ; *center panels*, TNF- α ; and *bottom panels*, IL-2. The panels labeled unstimulated indicate the background levels of cytokine expression in cells cultured in medium alone. The panels labeled peptide stimulated show the cytokine expression in SIV Gag p11C-stimulated lymphocytes, and the panels labeled PMA + Iono show the cytokine expression in PMA and ionomycin-stimulated lymphocytes. All histograms contained 30,000 gated CD3⁺CD8⁺ cells, and the percentage of monkey CD3⁺CD8⁺p11C tetramer⁺ T lymphocytes in the *upper left or right quadrants* is indicated.

induced IFN- γ expression in 80% (median; range 66–96%), TNF- α expression in 88% (median; range 78–98%), and IL-2 expression in 42% (median; range 21–47%) of the p11C tetramer⁺CD8⁺ T cells. The maximal potential cytokine secretion of these epitope-specific cells was determined by PMA and ionomycin stimulation. Most of these lymphocytes were able to produce IFN- γ (median 98%; range 95–100%) and TNF- α (median 90%; range 59–98%), while a lower percentage (median 54%; range 31–86%) was capable of IL-2 production.

Cytokine expression by Gag epitope-specific CD8⁺ T cells in monkeys infected with pathogenic SIV and SHIV isolates

We then analyzed the cytokine expression profile of CTL in a group of *Mamu-A*01*⁺ monkeys infected with pathogenic SIV or SHIV on day 150 postchallenge. These animals had high viremia and clear signs of progressive disease (Table I). A total of 62% (median; range 33–79%) of the p11C tetramer⁺CD8 $\alpha\beta$ ⁺ T cells expressed IFN- γ , and 66% (median; range 21–69%) expressed TNF- α , while only 10% (median; range 3–14%) expressed IL-2 following stimulation by Gag epitope peptide. Similarly, 92% (median; range 75–97%) of these same cells expressed IFN- γ and 67% (median; range 47–85%) expressed TNF- α , and 10% (median; range 4–24%) expressed IL-2 when stimulated by the polyclonal activators PMA and ionomycin (Fig. 3B). A statistical analysis using the Wilcoxon rank sum test with Bonferroni adjustments of *p* values demonstrated that the mean specific peptide-induced IL-2 and TNF- α production of this group of animals infected with pathogenic viral isolates differed significantly from the group infected with nonpathogenic/pathogenically attenuated isolates (*p* = 0.03) (Fig. 4). Therefore, the capacity of CD8⁺ CTL to produce IL-2 following both specific and nonspecific stimulation was substantially diminished in the peripheral blood of these chronically infected monkeys.

Analysis of cytokine expression in Gag epitope-specific CD8⁺ T lymphocytes in vaccinated monkeys challenged with SHIV-89.6P

Finally, we assessed cytokine production by CTL in a group of monkeys that were vaccinated and then challenged with the highly pathogenic SHIV-89.6P virus on day 150 postchallenge. These animals had been vaccinated with plasmid DNA constructs expressing HIV-1 89.6P Env (KB9) and SIVmac239 Gag augmented with either IL-2/Ig protein or an IL-2/Ig plasmid. Two further monkeys from this vaccination trial, animals that received a sham vaccine (KPB) or the DNA vaccines alone (811), are included in the cohort of monkeys evaluated as animals with progressive disease (Table I, Fig. 3B). Upon stimulation with Gag epitope peptide, 66% (median; range 51–76%) of the p11C tetramer⁺CD8 $\alpha\beta$ ⁺ T cells from this group of vaccinated and challenged animals expressed IFN- γ , 80% (median; range 78–90%) expressed TNF- α , and 27% (median; range 18–63%) expressed IL-2. The potent polyclonal stimulation resulting from PMA and ionomycin treatment induced 92% (median; range 86–96%) of the p11C tetramer⁺CD8 $\alpha\beta$ ⁺ T cells to produce IFN- γ , 91% (median; range 79–99%) to produce TNF- α , and 25% (median; range 13–46%) to produce IL-2 (Fig. 3C). The statistical comparison of this vaccinated group to the untreated animals that similarly received a pathogenic virus infection demonstrated a highly significant difference (*p* = 0.008) (Fig. 4). Thus, the cytokine expression profiles of CTL from these vaccinated and challenged animals were similar to those of the clinically healthy animals infected with nonpathogenic or pathogenically attenuated viruses. These data demonstrate that CTL of monkeys that have been effectively vaccinated before challenge with a highly pathogenic SHIV maintain their capacity to produce IL-2 and TNF- α in response to either Gag epitope-specific or nonspecific mitogen stimulation.

Table I. Clinical data of rhesus monkeys infected with nonpathogenic, pathogenically attenuated, or pathogenic virus isolates^a

Rhesus Monkey	Infecting Virus	Viral Load (RNA copies/ml)	CD4 Count (count/ μ l)
Infection with nonpathogenic or pathogenically attenuated virus			
206	SHIV-89.6	<500	977
403	SIVmac251(J5)	<500	859
556	SHIV-89.6	<500	1032
287	SHIV-89.6	<500	799
Infection with pathogenic virus			
N529	SIVsmE660	371,148	314
811	SHIV-89.6P	177,019	272
KPB	SHIV-89.6P	24,667	12
P967	SIVsmE660	2,646	67

^aRhesus monkeys were grouped into two cohorts. The animals infected with the nonpathogenic/pathogenically attenuated viruses had consistently low viral loads and nonprogressive disease and had been infected for >700 days. Animals infected with pathogenic virus had clinical signs of progressive disease, low CD4⁺ T lymphocyte counts and variable viral loads were at day 150 postchallenge.

Discussion

The inevitable failure of the immune system to contain the replication of HIV-1 in infected individuals has been ascribed, at least in part, to a progressive loss of functional CD8⁺ CTL. The etiology of this loss of CTL function is likely to be multifactorial. It has been shown that viral variants can emerge that are capable of escaping CTL recognition (28, 29). Other reports have described cytotoxic defects associated with deficits in perforin produced by CTL, a dysregulation in T cell costimulation, and a block in CTL maturation (1, 10, 30, 31). Finally, some studies have suggested that HIV-1-specific CD8⁺ CTL may be noncytotoxic because of a relative inability to produce IFN- γ (32). In fact, the ability of Ag-specific CD8⁺ CTL to produce IFN- γ following Ag stimulation has become a surrogate indicator of the functional status of these cells.

In this study, we demonstrate a statistically significant reduction in the ability of virus-specific CD8⁺ CTL to produce IL-2 and TNF- α in monkeys with progressive clinical disease (Fig. 4). A statistically significant decreased capacity of these cells to produce IFN- γ was not seen. These data suggest that rather than using IFN- γ production as an indicator of virus-specific CD8⁺ CTL fitness, it may be more useful to determine the capacity of these cells to produce IL-2 and/or TNF- α .

Other studies have also demonstrated a loss of the ability of virus-specific CD8⁺ CTL to produce IL-2 in this clinical setting (10). It has been suggested that this dysregulation in IL-2 production is associated with a lack of CD4⁺ T cell help, and that expansion of virus-specific CD8⁺ T lymphocytes in the absence of IL-2 can lead to the establishment of anergy in these cells (10, 33).

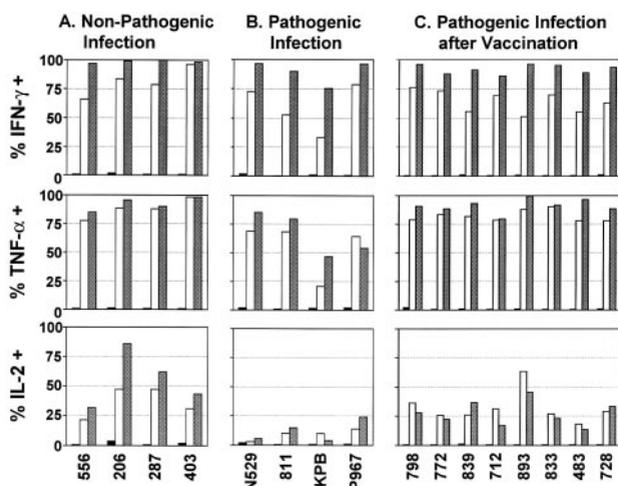


FIGURE 3. Cytokine expression profiles of CD3⁺ CD8⁺ p11C tetramer⁺ T cells in PBMCs from a cohort of rhesus monkeys infected with: *A*, nonpathogenic/pathogenically attenuated virus isolates; *B*, pathogenic virus isolates; or *C*, vaccinated with plasmid DNA constructs encoding HIV-1 89.6P Env (KB9) and SIVmac239 Gag together with either IL-2/Ig protein (798, 772, 839, 712) or an IL-2/Ig-encoding plasmid (893, 833, 483, 728) and then challenged with the highly pathogenic SHIV-89.6P virus isolate. PBMCs were cultured with media alone (■), SIV Gag p11C peptide (□), or PMA and ionomycin (▨). IFN- γ , TNF- α , and IL-2 expression is shown as the percent positive of the CD3⁺ CD8⁺p11C tetramer⁺ lymphocyte population.

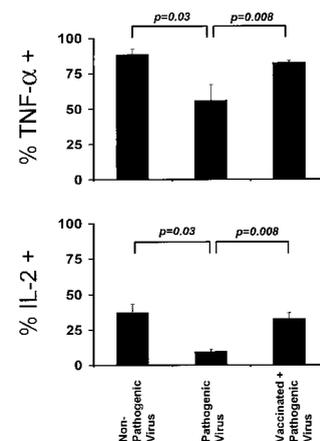


FIGURE 4. Statistical analyses of the mean cytokine expression by CTL from each group of animals, elicited by specific p11C peptide stimulation. The cytokine production by CTL from each group of monkeys was compared using a two-sided Wilcoxon rank sum test with Bonferroni adjustments of *p* values for the two major comparisons. Specific p11C peptide-induced IL-2 and TNF- α production differences between CTL from the animals infected with nonpathogenic (IL-2 = 36.8% (SD 6.4%), TNF- α = 88% (SD 4.2%)) and pathogenic (IL-2 = 9.2% (SD 2.0%), TNF- α = 55.5% (SD 11.6%)) virus isolates were highly significant (*p* = 0.03). The cytokine production by CTL from the vaccinated group of animals infected with pathogenic virus (IL-2 = 32.1% (SD 4.8%), TNF- α = 82.1% (SD 1.7%)) also differed significantly from that of the untreated animals similarly infected with pathogenic virus isolates (*p* = 0.008). A *p* value of <0.05 was considered significant.

Table II. Clinical data on vaccinated rhesus monkeys infected with SHIV-89.6P^a

Rhesus Monkey	Infecting Virus	Viral Load (RNA copies/ml)	CD4 Count (count/ μ l)
IL-2-Ig protein + DNA vaccines			
798	SHIV-89.6P	1323	888
772	SHIV-89.6P	1166	667
839	SHIV-89.6P	<500	720
712	SHIV-89.6P	<500	1085
IL-2-Ig plasmid + DNA vaccines			
893	SHIV-89.6P	651	1276
833	SHIV-89.6P	<500	858
483	SHIV-89.6P	<500	1554
728	SHIV-89.6P	719	968

^a Clinical status of the vaccinated group of rhesus monkeys on day 150 postchallenge. Animals were immunized with an HIV-gp120-encoding plasmid and an SIV-mac Gag encoding plasmid with IL-2/Ig delivered either as a protein or plasmid, prior to challenge with the pathogenic SHIV-89.6P isolate.

While we saw low IL-2 production by CD8⁺ CTL in the cohort of animals that had high viral loads and low CD4⁺ T lymphocyte counts, these data do not differentiate between these potential mechanisms. Thus, a loss of the capacity of CTL to produce IL-2 may reflect a loss of the CD4⁺ T cell help needed to expand virus-specific CD8⁺ CTL or a specific defect in the functional repertoire of the virus-specific CD8⁺ CTL population.

Importantly, the virus-specific CD8⁺ CTL from all the animals that were clinically healthy following infection with pathogenically attenuated or nonpathogenic SIV and SHIV isolates produced normal levels of IL-2 and TNF- α . In addition, the monkeys that were vaccinated before infection also showed normal levels of IL-2 and TNF- α production by their virus-specific CD8⁺ CTL. These animals were immunized with DNA vaccines augmented by IL-2/Ig protein or IL-2/Ig plasmid before challenge and exhibited significant control of viremia and prevention of clinical disease progression (Table II) (27). These vaccinated animals had CTL that exhibited levels of IL-2 and TNF production that were significantly higher than the levels observed in the CTL of typical progressors ($p = 0.008$), two of which were similarly infected with SHIV-89.6P. In fact, the levels of cytokine production in the vaccinated animals were comparable with the levels observed in the clinically healthy animals infected with nonpathogenic/pathogenically attenuated virus isolates. Thus, effective vaccination of rhesus monkeys before a pathogenic viral challenge preserved their capacity to produce cytokines in response to specific Ags and established a phenotype comparable with the animals infected with pathogenically attenuated and nonpathogenic viruses.

In conclusion, we have demonstrated cytokine dysregulation by virus-specific CD8⁺ T lymphocytes during progressive SIV/SHIV infection that can be reversed by prior vaccination. The use of IFN- γ production may not be as sensitive as the determination of IL-2 and TNF- α production as an indicator of virus-specific CD8⁺ T cell function. The development of in vitro assays such as an ELISPOT for quantification of the IL-2 and TNF- α responses in virus-specific T cell populations may prove useful in addition to assays for IFN- γ production to help determine the functional capacity of HIV-1-specific CTL.

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