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Differences Between T Cell Epitopes Recognized After Immunization and After Infection[1]

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Evidence suggests that cellular immune responses play a crucial role in the control of HIV and SIV replication in infected individuals. Several vaccine strategies have therefore targeted these CD8^{+} and CD4^{+} responses. Whether vaccination induces the same repertoire of responses seen after infection is, however, a key unanswered question in HIV vaccine development. We therefore compared the epitope specificity induced by vaccination to that present postchallenge in the peripheral blood. Intracellular cytokine staining of PBMC stimulated with overlapping 15/20-mer peptides spanning the proteins of SIV were measured after DNA/modified vaccinia Ankara vaccination of eight rhesus macaques. Lymphocytes from 8 animals recognized a total of 39 CD8 epitopes and 41 CD4 epitopes encoded by the vaccine. T cell responses were again monitored after challenge with SIVmac239 to investigate the evolution of these responses. Only 57% of all CD8^{+} T cell responses and 19% of all CD4^{+} T cell responses present after vaccination were recalled after infection as measured in the peripheral blood. Interestingly, 29 new CD8 epitopes and 5 new CD4 epitopes were recognized by PBMC in the acute phase. These new epitopes were not detected after vaccination, and only some of them were maintained in the chronic phase (33% of CD8 and no CD4 responses). Additionally, 24 new CD8 epitopes and 7 new CD4 epitopes were recognized by PBMC in the chronic phase of infection. The repertoire of the immune response detected in the peripheral blood after immunization substantially differed from the immune response detected in the peripheral blood after infection. *The Journal of Immunology,* 2002, 169: 4511–4521.

Many studies have indicated the importance of the cellular immune response, especially the CTL, in controlling the replication of the immunodeficiency viruses HIV and SIV. Plasma viral concentrations in infected individuals correlate inversely with the frequency of CTL present (1); the removal of CD8^{+} cells from SIV-infected monkeys leads to an increase in viral replication (2–4); and the presence of virus-specific CTL results in selection of mutant viruses, which are no longer recognized by these CTL (5–9). CTL may even be able to protect from infection with HIV (10–12). CD4^{+} T cells, in contrast, are important in maintaining a good CD8 (CTL) response (13, 14). The presence of strong CD4^{+} responses in HIV-positive long term nonprogressors has been taken as evidence for the important role of these lymphocytes in control of virus replication (15). Therefore, many vaccine trails are now focusing on the induction of virus-specific cellular immune responses.

Each individual responds against a different set of epitopes as determined by that individual's MHC class I and class II molecules. Therefore, to evaluate experimental vaccines designed to induce CD8^{+} and CD4^{+} cellular responses, it is important to measure the entire repertoire of responses against the virus proteins used in the vaccine. Although the advent of tetramer technology (16) has made it possible to enumerate epitope-specific T cell populations (17–20), this method requires prior knowledge of the T cell epitope and the restricting allele. Another recent technological advance for monitoring virus-specific T cell immune responses is intracellular cytokine staining (ICS[4]; Refs. 21 and 22). This technique has an advantage over tetramer technology in that it does not require prior knowledge of the T cell epitope or restricting allele. Peptides spanning entire viral proteins can be used to stimulate PBMC from vaccinated or infected individuals (23), and peptide-specific T cells, which are producing cytokines, are detected by flow cytometry. In addition, ICS can also be used to map peptide-specific responses (23–25).

SIV infection in rhesus macaques is similar to HIV infection in humans and is an excellent animal model for HIV vaccine research. Both immunizations and infections can be controlled in the laboratory setting, making this animal model ideally suited for comparing the epitope specificity of immune responses induced by vaccination to those engendered after viral challenge. The aim of this study was to identify and characterize SIV-derived epitopes for both CD8^{+} and CD4^{+} T cells elicited by vaccination and determine whether they were recalled after challenge. We reasoned that vaccine-primed responses recalled postchallenge may be important for control of viral replication. We therefore characterized

the cellular immune responses against all SIV proteins after immunization and also after infection. Our results suggest that the immune responses detected in the PBMC induced by immunization substantially differ from the immune responses detected in PBMC engendered by infection. This suggests that measurement of responses in the peripheral blood may not be the best way to study HIV-specific vaccines.

Materials and Methods

Animals

Rhesus macaques (*Macaca mulatta*) were maintained in accordance with the National Institutes of Health Guide to the Care and Use of Laboratory Animals and under the approval of the University of Wisconsin Research Animal Resource Center review committee.

Peptides

Overlapping peptides (20-mers, 15-mers) were synthesized by Chiron (Raleigh, NC) or the Natural and Medical Science Institute (University of Tubingen, Tubingen, Germany) based on SIVmac239 protein sequences, with the exception of Pol peptides, which corresponded to the SIVmac251 sequence. Lyophilized peptides were resuspended in PBS with 10% DMSO (Sigma-Aldrich, St. Louis, MO). Consecutive 20-mer and 15-mer peptides overlap by 10 or 11 aa, respectively. Pools of peptides contained 10 peptides at a final concentration of 1 mg/ml/peptide.

PBMC

PBMC were separated from whole heparinized blood by Ficoll-diatrizoate (Histopaque; Sigma-Aldrich) density gradient centrifugation. The PBMC were either used immediately or stored at $-180°C$ in liquid nitrogen. PBMC were cultured in RPMI 1640 supplemented with 15% FCS, 2 mM L-glutamine, 25 mM HEPES, 25 μM 2-ME, 50 μg/ml streptomycin, 50 U/ml penicillin (R15 medium) containing 20–100 IU/ml recombinant IL-2 (Proleukin; Chiron, Emeryville, CA).

B-lymphoblastoid cell lines (B-LCL)

Rhesus monkey B-LCL were generated as described previously (26) by incubating PBMC with *Herpes papio* produced by S594 cells. All B-LCL lines were cultured in RPMI 1640 supplemented with 10% FCS, 2 mM L-glutamine, 25 mM HEPES buffer, 25 μM 2-ME, 50 μg/ml streptomycin, and 50 U/ml penicillin (R-10 medium).

DNA/modified vaccinia Ankara (MVA) vaccinations

Animals were immunized 10 times with DNA using the PowderJect XR1 device (PowderJect Vaccines, Madison, WI) at intervals of 4–9 wk, as described elsewhere (27). Briefly, four vaccine plasmids encoding SIV Gag, Pol, Env, Vif, Vpr, Vpx, Tat, Rev (pSIV17E-Fr gag-pol-env), SIV Nef (pSIVNef-TPA and pSIVNef), and SIV Rev (pSIVrev) were coadministered and therefore included all the proteins of SIV. All protein sequences were derived from the macrophage-tropic clone SIVmac17E-Fred except Rev, which was derived from both the SIVmac239 and SIV17E-Fr sequences (28–30). The macrophage-tropic clone SIVmac17E-Fred is closely related to SIVmac239 (28, 29). The construction of these DNA vectors has been described elsewhere (27). Approximately 1 year after the last DNA vaccination, all animals of the vaccine group were inoculated twice with rMVA virus vaccines within a 13-wk interval (27). The animals received 1 × 10^8 infectious U of each MVA vector vaccine, encoding SIVmacJ5 (31) Gag/Pol, Env, Nef, Rev, and Tat (no MVA available expressing Vif, Vpr, or Vpx) delivered intradermally (into five sites) and also intrarectally (27). No side effects or lesions were associated with the inoculations.

In vitro stimulation of PBMC

To generate peptide-specific CD8 and CD4 T cell lines, fresh PBMC were stimulated in vitro with peptide-pulsed, autologous B-LCL as stimulator cells. In detail, stimulator cells were generated by incubation of 5 × 10^6 autologous B-LCL with 0.1–0.5 μM peptide at 37°C in a 5% CO$_2$ humidified atmosphere. After 1.5 h, the cells were gamma-irradiated (3000 rad), washed twice with R-15 medium, and added to 5 × 10^6 fresh autologous PBMC in R-15 medium. At day 0, 2.5 kU/ml rhIL-7 (R&D, Minneapolis, MN) were added to the culture medium. After 2 days, 20 U/ml recombinant IL-2 (Proleukin) were added, and the cultures were fed afterward every second day with 100 U/ml IL-2-containing R-15 medium. At day 7, CD8β^+ cells and CD4$^+$ cells were separated using the Miltenyi Biotec

(Auburn, CA) MiniMACS system. Briefly, the 7-day-old in vitro stimulated culture were Ficoll purified (Ficoll-diatrizoate; Histopaque), washed twice with FACS buffer (PBS, 2% FBS), and incubated with 6 μl anti-CD8β-PE (Immunotech, Westbrook, ME) and 10 μl anti-CD4-FITC (clone SK3; BD Biosciences, San Jose, CA) for 30 min at 6°C in MACS buffer (FACS buffer, 2 mM EDTA). Afterward, the PBMC were incubated with anti-FITC beads (Miltenyi Biotec) and CD4$^+$ cells were enriched using MS$^+$ columns for MiniMACS (Miltenyi Biotec) according to the manufacturer's protocol. The remaining PBMC, depleted of CD4$^+$ cells, were then incubated with anti-PE-beads (Miltenyi Biotec), and the CD8β^+ cells were enriched in the same manner as the CD4$^+$ cells. These CD8β^+ cells and CD4$^+$ cells, now in separate cultures, were again stimulated in vitro using peptide-pulsed, autologous B-LCL as stimulator cells. After a total of 14 days of in vitro stimulation, the cells were used as effectors in ICS to test for peptide-specific cells.

ICS with fresh PBMC

PBMC were separated from whole heparinized blood by Ficoll-diatrizoate density gradient centrifugation. PBMC (1 × 10^6) were incubated with either staphylococcal enterotoxin B (10 μg/ml; Sigma) as a positive control or pools of ten 15/20-mer peptides together with 0.5 μg anti-CD28 (clone L293) and 0.5 μg anti-CD49d (clone 9F10; BD PharMingen, San Diego, CA) in a total volume of 200 μl R-10 (see above). Anti-CD28 and anti-CD49d Abs were added to provide optimal costimulation (22). After 1.5 h at 37°C 10 μg/ml brefeldin A were added, and the cells were further incubated for 5 h at 37°C. Brefeldin A inhibits the export of proteins from the endoplasmic reticulum and results, therefore, in the intracellular accumulation of cytokines, which would otherwise be secreted. Cells were washed twice with 1 ml FACS buffer (PBS plus 2% FCS) and then stained with 6 μl CD8α-PerCP (clone SK1; BD Biosciences) and 4 μl CD4-APC (clone SK3; BD Biosciences) in 100 μl FACS buffer for 40 min. After two washes with 1 ml FACS buffer, the cells were fixed with 2% paraformaldehyde-PBS solution overnight at 4°C. The cells were then washed once with FACS buffer, treated with permeabilization buffer (0.1% saponin in FACS buffer) for 10 min at room temperature, washed once more with 0.1% saponin buffer, and resuspended in 100 μl 0.1% saponin buffer. Then, 1 μl anti-human IFN-γ-FITC mAb (clone 4S.B3; BD PharMingen, San Diego, CA) and either 6 μl anti-CD69-PE (clone L78; BD Biosciences) or 1 μl anti-human TNF-α-PE mAb (clone Mab11; BD PharMingen) were added. After 50 min of incubation at room temperature, the cells were washed twice with 0.1% saponin buffer, with a 10-min incubation before the last spin, and then fixed with 2% paraformaldehyde-PBS. Samples were stored in the dark at 4°C, and acquisition of 100,000–200,000 lymphocyte-gated events was performed on a FACSCalibur flow cytometer (BD Biosciences) and analyzed with FlowJo software (Tree Star, San Carlos, CA). The background level of IFN-γ staining in PBMC (induced by a control influenza peptide SNEGSYFFG) varied from animal to animal but was typically below 0.05% in the CD8$^+$ lymphocytes and below 0.02% in the CD4$^+$ lymphocytes. Only samples in which the IFN-γ staining was at least twice that of the background or where there was a distinct population of IFN-γ bright positive cells (also positive for CD69 or TNF-α) were considered positive. All values are reported after subtraction of the background level staining.

Challenge with molecularly cloned SIVmac239/nef-open

Nine weeks after the last MVA boost, all animals were challenged intrarectally with a molecularly cloned virus, SIVmac239/nef-open (30), using a dose of ~10 intrarectal monkey infectious dose 50% (32), as described elsewhere (27).

Viral sequence analysis

Viral sequencing was performed as previously described (33). Briefly, SIV RNA was extracted from EDTA plasma using the QIAmp viral RNA kit (Qiagen, Valencia, CA). Amplified cDNA spanning recognized CTL epitopes was generated in a 1-step RT-PCR (Qiagen) using sequence-specific primers for reverse transcription and amplification. The primers used have been described previously (33). The RT-PCR amplicons were directly purified using the QIAquick method (Qiagen), and the purified products were directly sequenced on an ABI 377 automated sequencer (Applied Biosystems, Foster City, CA). The sequence traces were analyzed as previously described using Sequencher version 4.1 for Macintosh (Genecodes, Ann Arbor, MI) and aligned to SIVmac239 with MacVector 7.0 Trial Version (Accelerys, San Diego, CA). The dominant viral sequence at each nucleotide site is shown; mixed bases with a secondary peak height of 50% of the maximal peak are indicated using standard International Union of Biochemistry nomenclature.

Results

Mapping CD8 and CD4 responses in fresh PBMC from monkeys boosted with rMVA

We used pools of peptides spanning the proteins Gag, Pol, Env, Nef, Tat, and Rev (10 overlapping 15/20-mer peptides per pool) to stimulate fresh PBMC in 8 rhesus macaques immunized with a DNA prime/MVA boost vaccine. Two of the animals were Mamu-A*01 positive. PBMC were obtained from all animals 1 wk after the boost with rMVA, a time point when the frequency of virus-specific T cells is expected to be high (34). Incubation of PBMC with pools of peptides resulted in the stimulation of either CD8^{+} (Fig. 1A) or CD4^{+} lymphocytes (Fig. 2A) to produce IFN-γ as detected by ICS. Peptide pools that yielded a positive result were then subdivided into the individual peptides and tested again by ICS using fresh PBMC obtained 2 wk after the MVA boost. In most cases, one or two overlapping peptides (Figs. 1B and 2B) were identified that were responsible for the positive reaction of a pool. Using this approach, we detected responses against a total of 34 CD8 epitopes (Table I) and 37 CD4 epitopes (Table II) encoded by the vaccine. Although the frequencies of these responses were quite low, they were easily identified based on the presence of a bright IFN-γ-positive population of cells, also positive for CD69 (Figs. 1B and 2B). In both Mamu-A*01-positive animals (80035 and 96135), we detected responses against two epitopes (Gag 45/46 and Tat 10/12) that correspond to the previously described Mamu-A*01-restricted epitopes, Gag$_{181-189}$CM9 and Tat$_{28-35}$SL8 (8, 35). Both tetramer staining and ICS revealed similar frequencies for these two Mamu-A*01-restricted epitopes (not shown), confirming our previous finding that our ICS was as sensitive as the tetramer staining (34, 36). With the exception of animal 87081, all animals recognized at least one CD8 epitope (Table I) and at least two CD4 epitopes post vaccination (Table II). Animal 93062 recognized 11 CD8 epitopes after the first rMVA boost (Table I).

A second recombinant MVA booster immunization did not result in an efficient boost of the cell-mediated immune response

We administered a second booster immunization with rMVA 13 wk after the first MVA boost and again analyzed the total T cell response by ICS. Unlike that seen after the first boost, the second booster immunization with the rMVA did not efficiently boost the SIV-specific immune response in most animals. In addition, after the second MVA boost we were able to detect only a subset of the epitope-specific responses that were detected after the first MVA boost (Tables I and II). However, if the cells were restimulated in vitro to increase the sensitivity of the ICS analysis, many CD8 and CD4 epitopes that were detected after the first MVA boost could be readily detected after the second MVA boost (Tables I and II, gray boxes). Despite the failure of the second MVA to boost all previously identified responses, five new CD8^{+} and four new CD4^{+} responses not detected after the first MVA were detected in these animals (Tables I and II). Preexisting vaccinia immunity at the time of the second rMVA boost may have reduced the effectiveness of the second rMVA boost (37, 38).
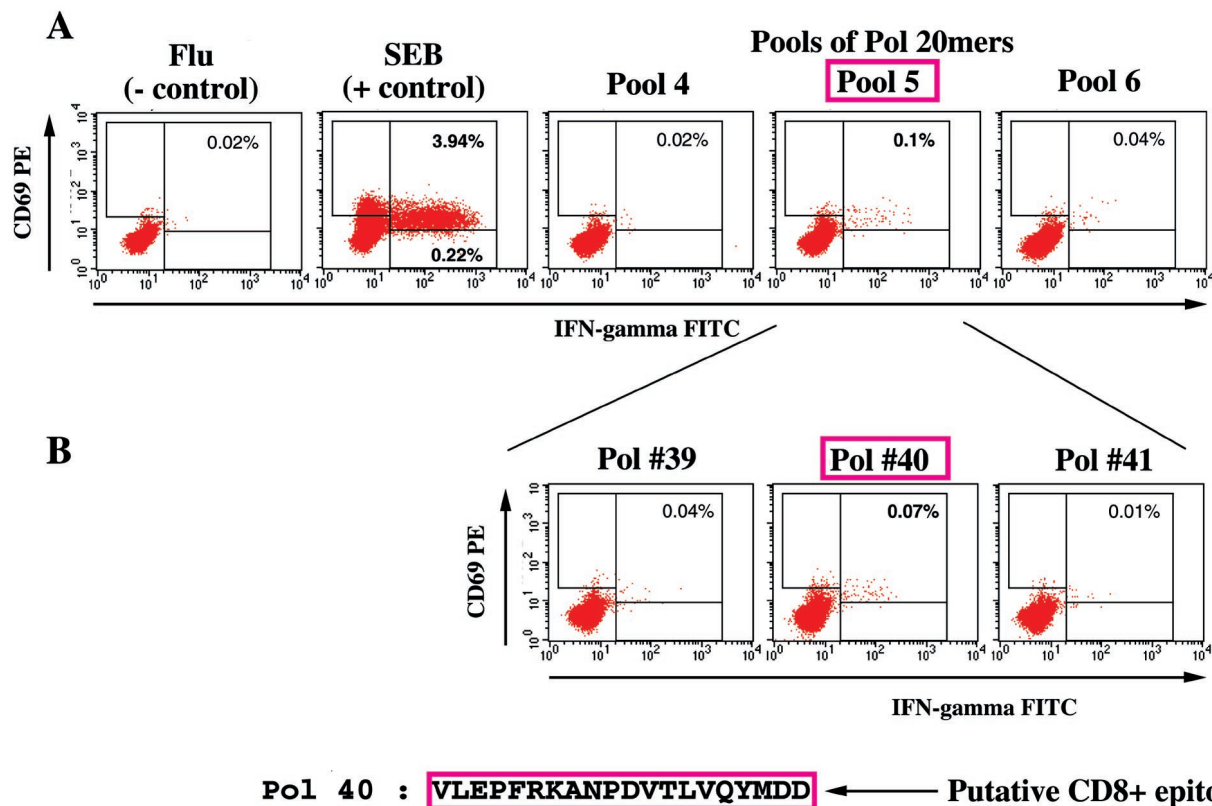
FIGURE 1. Example of CD8^{+} T cell epitope mapping in an animal after the rMVA boost. One week after the boost with recombinant MVA-expressing SIV proteins, PBMC were isolated and tested in ICS against pools of peptides (A) as described in *Materials and Methods*. Any peptide contained in a pool that induced a positive response, as detected through the presence of IFN-γ^{+}/CD69^{+} cells (see Pol pool 5), was then tested with PBMC obtained in the second week after the immunization (B). Background staining in the CD8^{+} lymphocytes induced by the Flu peptide (specific for this animal and time point) was 0.02%. Therefore, only values >0.04% were considered positive (see *Materials and Methods*). The 20-mer peptide Pol 40 (B) induced a positive IFN-γ production in the CD8-positive lymphocytes and therefore contains a putative CD8 epitope. This is an example of an epitope that was no longer detectable after infection with SIVmac239. SEB, Staphylococcal enterotoxin B.
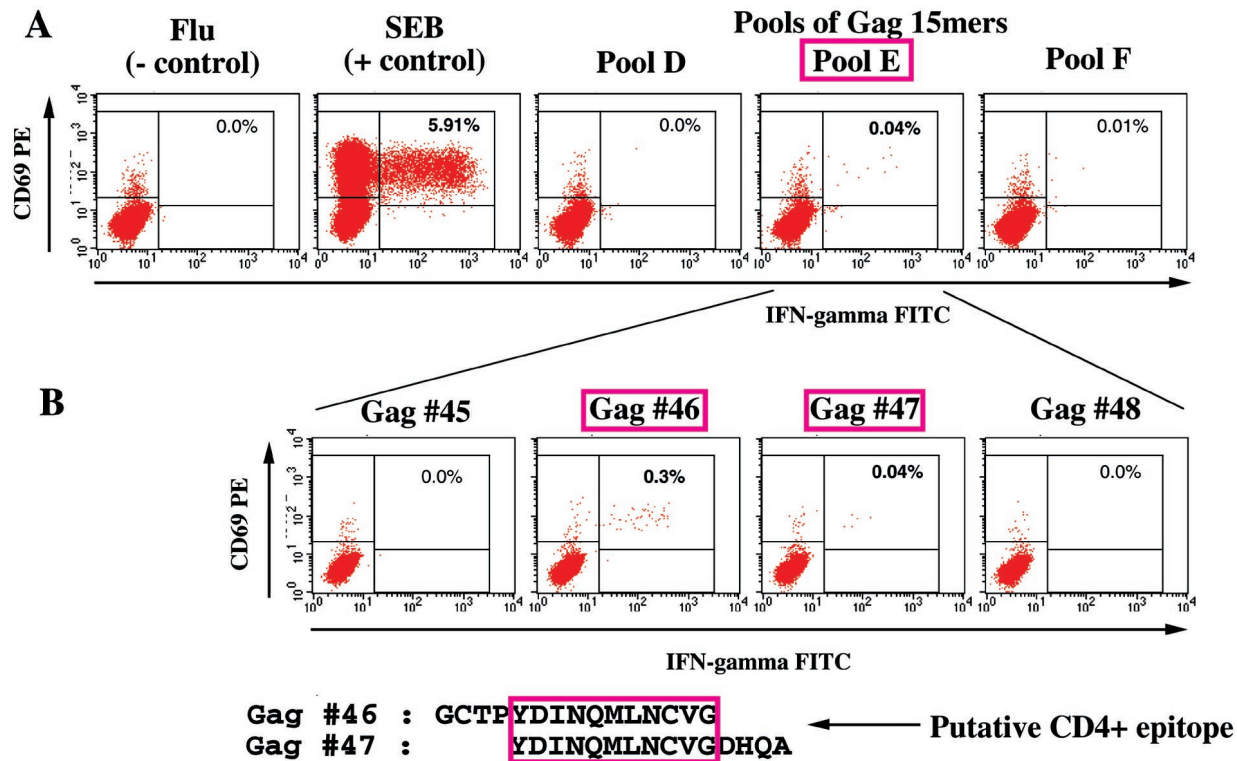
FIGURE 2. Example of CD4+ T cell epitope mapping in an animal after the rMVA boost. As described in Fig. 1, PBMC were isolated and tested in ICS against pools of peptides (*A*). Any peptide contained in a pool, which induced a positive response, as detected through the presence of IFN-γ+/CD69+ cells (see Gag pool E), was then tested with PBMC obtained in the second week after the immunization (*B*). Background staining in the CD4+ lymphocytes induced by the Flu peptide (specific for this animal and time point) was 0.00%. In this case, only values above 0.01% were considered positive (see *Materials and Methods*). The 15-mer peptides Gag 46/47 (*B*) stimulated CD8-positive lymphocytes and, therefore, contain a putative CD4 epitope. This is an example of an epitope that was no longer detectable after infection with SIVmac239. The higher IFN-γ-staining with the individual peptide in comparison with the pool is most likely the result of the different time points these responses were detected (pools were tested at wk 1 and the individual peptides then at wk 2 postvaccination). When done at the same time point, pools and individual peptides induce equivalent stimulation (not shown; see Ref. 84).

Evolution of the CD8 cellular immune response in PBMC after infection with SIVmac239

Nine weeks after the last MVA boost, all animals were challenged intrarectally with molecularly cloned SIVmac239/nef open. The outcome of this challenge, viral loads and the correlations with the overall immune responses are presented in detail elsewhere (27). All animals became infected. However, all vaccinated animals demonstrated a significantly reduced peak of the plasma virus load as measured by real time PCR (27). To determine whether the cellular immune response induced by the DNA prime/MVA boost was recalled after infection, we performed ICS 2 and 3 wk postchallenge with pools and individual peptides spanning all nine SIV proteins encoded by the virus. Additionally, to follow the evolution of the response over the time course of infection, whole genome ICS was also performed 12 and 13 wk postchallenge. Most of the previously identified epitopes in each animal were included as individual peptides at all time points.

Interestingly, not all CD8 responses mapped after vaccination were recalled postinfection. Several epitopes remained undetectable when measured during either the acute phase (wk 2/3) or the chronic phase (wk 12/13) of infection (Table I). During the acute phase of infection (2 or 3 wk postchallenge), a recall response was detected against only 16 of 28 vaccine-induced CD8+ T cell responses that were tested (a total of 39 epitopes were detected after vaccination). However, 24 new CD8 epitopes that were not detected postvaccination were recognized in the postchallenge acute phase. In addition, five de novo CD8 epitopes were recognized in Vif or Vpr (Table I). These two proteins were encoded by the DNA

vaccine used for priming but they were not included in the MVA boost. Of the 45 CD8 responses detected during the acute phase (vaccine induced and new responses after infection), 39 were again tested by ICS during the chronic phase, at wk 12/13. Among these 39, only 16 epitopes were maintained in the chronic phase. Finally, 24 new CD8 epitopes were detected during the chronic phase that were not detected either postvaccination or during the acute phase.

Evolution of the CD4 cellular immune response in the PBMC after infection with SIVmac239

After vaccination, CD4 responses were detected against 41 epitopes. However, during the acute phase of infection, a recall response was detected against only a few of these epitopes (Table II). Only animals 93062 and 87081 had recall CD4 responses in the acute phase of infection (2 or 3 wk postinfection), and a recall response was detected against only 7 of the 37 vaccine-induced CD4+ T cell responses tested at this time point (a total of 41 epitopes were detected after vaccination). As with the CD8 responses, we detected CD4 responses against five new epitopes (animals 87081 and 93062, Table II) that had not been detected after vaccination. These new CD4 responses were not maintained into the chronic phase. However, animals 87081, 80035, and 81035 developed new CD4 responses in the chronic phase against epitopes that had not been detected after vaccination or during the acute phase (Table II). In these three animals, a total of seven new CD4 epitopes were recognized in the chronic phase of infection.

Table I. *Frequencies of CD8 responses in rhesus macaques after immunization and after challenge with SIVmac239*

Animal	Peptide	Sequence [a]	post 1. MVA	post 2. MVA	Acute[b] Infection	Chronic[c] Infection
97073	Gag 65	PIPVGNIYRRWIQLG [d]	0.017	-	0.01	
	Gag 66	GNIYRRWIQLGLQKC		-[e]	0.03	
	Env 85-87	ATETLAGAWGDLWETLRRGGRWILAIPRRIRQGLELTLL				0.06
	Tat 14/15	ANLGEEILSQLYRPLEACY	g		0.32	0.05
	Tat 16/17	SQLYRPLEACYNTCYCKKC	-		0.19	0.05
	Vpr 17	LQRALFMHFRGGCIH			-	0.03
	Vpr 18	LFMHFRGGCIHSRIG			0.3	
	Vpr 19	FRGGCIHSRIGQPGG				0.02
	Vpr 20	CIHSRIGQPGGGNPL			-	
93062	Gag 44	PGFQALSEGCTPYDI	0.1		n.t.	n.t.
	Gag 45	ALSEGCTPYDINQML	0.09		n.t.	n.t.
	Gag 46	GCTPYDINQMLNCVG	0.03		0.1	-
	Gag 47	YDINQMLNCVGDHQA	0.08			-
	Gag 50	HQAAMQIIRDIINEE	0.04		n.t.	-
	Gag 61/62	TSSVDEQIQWMYRQQNPIP	-		1.91	-
	Gag 63/64	QWMYRQQNPIPVGNIYRRW	-		1.83	-
	Gag 67/68	RRWIQLGLQKCVRMYNPTN	-		0.69	-
	Gag 69	QKCVRMYNPTNILDV	0.155	0.06	-	-
	Gag 70	RMYNPTNILDVKQGP	0.34	0.17	1.06	0.18
	Gag 91/92	KARLMAEALKEALAPVPIP	-		0.64	-
	Gag 95	PIPFAAAQQRGPRKP	0.13	0.05	0.31	0.03
	Gag 96	AAAQQRGPRKPIRCW	0.25	0.03	-	0.07
	Gag 97/98	QRGPRKPIKCWNCGKEGHS	-		1.25	-
	Gag 117	DPAVDLLKNYMQLGK	0.13		0.11	-
	Gag 118	DLLKNYMQLGKQQRE	-			-
	Gag119/120	NYMQLGKQQREKQRESREK	-		0.25	-
	Env 31/32	LNKYYNLTMKCRRPGNKTVLPVTIMSGLVF			0.76	-
	Env 35/36	QAAWCWPGGKWKDAIKEVKQTIVKHPRYTGT			1.01	-
	Env 37/38	IVKHPRYTGTNNTDKINLTAPGGGDPEVTF			0.1	-
	Env 41	YCKMNWFLNWVEDRNTANQK	0.1	-	-	n.t.
	Env 43	PKEQHKRNYVPCHIRQIINT	0.03	-	-	-
	Env 44	PCHIRQIINTWHKVGKNVYL	0.03	-		n.t.
	Env 45	WHKVGKNVYLPPREGDLTCN	0.04	-	0.46	-
	Env 46	PPREGDLTCNSTVTSLIANI	-	-		-
	Env 82	RIREVLRTELTYLQYGWSYF	0.08	-	0.04	-
	Env 83	TYLQYGWSYFHEAVQAVWRS	0.03	-		-
	Rev 22	QQLLALADRIYSFPD	0.08	-	-	-
	Vif 10	QKVCYVPHFKVGWAW				2.3
87081	Gag 11	LDRFGLAESLLENKE				0.01
	Gag 17	APLVPTGSENLKSLY				0.02
	Gag 61	TSSVDEQIQWMYRQQ	-		0.04	-
	Gag 64	RQQNPIPVGNIYRRW	-		0.12	-
	Gag 65	PIPVGNIYRRWIQLG	-		0.08	-
	Gag 70	RMYNPTNILDVKQGP	-		0.26	0.07
	Gag 71	PTNILDVKQGPKEPF			0.03	-
	Pol 105	PGELLWKGEGAVILKVGTDI				0.3
	Nef 4	ISMRRSRPSGDLRQR				0.05
	Nef 6	RSRPSGDLRQRLLRA				0.07
	Rev 12	NPYPTGPGTANQRRQ	-		0.15	0.1
	Vpx 18	YRYLCLIQKALFMHC				0.017
	Vpx 19	CLIQKALFMHCKKGC				0.03
87082	Gag 17	APLVPTGSENLKSLY	0.19		0.11	-
	Gag 18	PTGSENLKSLYNTVC	0.06		n.t.	-
	Gag 28	LVVETGTTETMPKTS	0.05		-	-
	Gag 43	AEVVPGFQALSEGCT	0.02		n.t.	n.t.
	Gag 50	HQAAMQIIRDIINEE	-		0.12	-
	Nef 37	YRKQNMDDIDEEDDD	0.02		-	-
	Nef 48	TMSYKLAIDMSHFIK	0.01		0.04	0.05
	Nef 63	PDWQDYTSGPGIRYP	0.1		n.t.	0.29
	Nef 64	DYTSGPGIRYPKTFG	0.13		0.58	0.29
	Env 72	VQMLAKLRQGYRPVFSSPPS			0.02	-
	Env 73	YRPVFSSPPSYFQQTHIQQD			0.01	-
	Vif 32	SKNFWTDVTPNYADI			0.45	0.12
	Vif 34	WTDVTPNYADILLHS			0.67	0.1
80035	Gag 1	MGVRNSVLSGKKADE	-	0.08	-	-
	Gag 45	ALSEGCTPYDINQML	0.1	0.03	n.t.	n.t.
	Gag 46	GCTPYDINQMLNCVG	0.2	0.09	0.45[i]	2.5[i]
	Pol 41-44	GPINTKEYKNVEIEVLGKRIKGTIMTG			0.11	n.t.
	Env 33-36	PVTIMSGLVFHSQPINDRPKQAWCWPGGKWKDAIKEVKQTIVKHPRYTGT			0.08	-
	Nef 25	GLSSLSCEGQKYNQG	0.18		n.t.	-
	Nef 28/29	GQKYNQGQYMNTFWRNPAE	-			0.22
	Nef 42-48	VGVSVRPKVPLRTMSYKLAIDMSHFIK		-	0.83	n.t.
	Nef 49-52	KLAIDMSHFIKEKGGLEGIYYSARRHR		-	0.72	n.t.
	Nef 54	EGIYYSARRHRILDI	0.35	-	4.11	-
	Nef 56-60	YSARRHRILDIYLEKEEGIIPDW	-			n.t.
	Tat 8	ERSSCISEADASTPE			n.t.	-
	Tat 10	CISEADASTPESANL	0.62	0.9	n.t.	0.28
	Tat 12	ADASTPESANLGEEI	0.61	0.7	9.48	0.2
	Tat 13	TPESANLGEEILSQL	0.1		n.t.	-
	Tat 19	TCYCKKCCYHCQFCF	0.07	-	n.t.	n.t.
	Tat 20	KKCCYHCQFCFLKKG	0.14	0.09	n.t.	0.2
	Tat 21	YHCQFCFLKKGLGIC	0.08	0.37	n.t.	-
	Tat 22	FCFLKKGLGICYEQS	0.35	-	n.t.	0.1
	Tat 23	KKGLGICYEQSRKRR	-	-	n.t.	-
	Tat 25	EQSRKRRRTPKKAKA	-	0.16	n.t.	n.t.
	Rev 2-6	MSNHEREEELRKRLRLIHLLHQTINPY	-	-	1.04	-
	Rev 8-14	IHLLHQTINPYPTGPGTANQRRQRKRR	-	-	0.29	-
	Rev 16-21	TANQRRQRKRRWRRRWQQLLALADRIY	-	-	0.34	-
	Vif 26/28	WLSTYAVRITWYSKNFWTD			0.06	0.1
	Vif 30-36	ITWYSKNFWTDVTPNYADILLHSTYFP			0.34	n.t.
	Vif 34/36	WTDVTPNYADILLHSTYFP			n.t.	0.02
	Vif 37/38	ADILLHSTYFPCPTAGEVR			-	0.07
	Vif 49/51	FPRAHKYQVPSLQYLALKVVSDV				0.06
	Vif 68-72	GGKPPTKGANFPGLAKVLGILA			0.08	-
	Vpx 3/4	PPGNSGEETIGEAFEWLNR				0.03
	Vpx 7/8	LNRTVEEINREAVNHLPRE				0.07

Animal	Peptide	Sequence [a]	post 1. MVA	post 2. MVA	Acute[b] Infection	Chronic[c] Infection
81035	Gag 47	YDINQMLNCVGDHQA				0.02
	Env 41	YCKMNWFLNWVEDRNTANQK	0.03		-	-
	Nef 38	NMDDIDEEDDDLVGV				0.03
	Nef 48	TMSYKLAIDMSHFIK				0.05
	Nef 50	DMSHFIKEKGGLEGI				0.07
	Nef 51	FIKEKGGLEGIYYSA				0.04
	Nef 54	EGIYYSARRHRILDI				0.01
	Nef 56	YSARRHRILDIYLEK				0.09
	Nef 58	RHRILDIYLEKEEGI				0.01
	Vpx 8	VEEINREAVNHLPRE				0.13
	Vpx 9	NREAVNHLPRELIFQ				0.05
96135	Gag 21	NTVCVIWCIHAEEKV	0.08		n.t.	n.t.
	Gag 42	KKFGAEVVPGFQALS	-	0.08	-	-
	Gag 45	ALSEGCTPYDINQML	0.12	0.05	n.t.	n.t.
	Gag 46	GCTPYDINQMLNCVG	0.27	0.08	3.1	0.7
	Pol 6	CYGQMPRQTGGFFRPWSMGK	-		0.04	-
	Pol 40	VLEPPRKANPDVTLVQYMDD		0.03	-	-
	Pol 75	EVQLGIPHPAGLAKR			0.07	0.1
	Env 10	WQLFETSIKPCVKLSPLCIT	0.05		n.t.	n.t.
	Env 55	SAMGAASL7LTAQSRTLLAG	0.17		-	-
	Nef 31	YMNTPWRNPAEEREK				0.1
	Nef 36	EKLAYRKQNMDDIDE				0.15
	Nef 49	KLAIDMSHFIKEKGG				0.058
	Nef 54	EGIYYSARRHRILDI				0.23
	Nef 65	GPGIRYPKTFGWLWK				0.09
	Rev 4	ELRKRLRLIHLLHQT				0.07
	Tat 10	CISEADASTPESANL	0.17	0.07	n.t.	n.t.
	Tat 12	ADASTPESANLGEEI	0.15	0.06	3	0.26
	Tat 14	ANLGEEILSQLYRP	-	0.04	n.t.	n.t.
	Tat 15	EEILSQLYRPLEACY	0.2	0.06	-	0.06
	Tat 16	SQLYRPLEACYNTCY	-	0.02	-	-
	Tat 20	KKCCYHCQFCFLKKG	-	-	n.t.	0.19
	Tat 21	YHCQFCFLKKGLGIC	-	-	n.t.	0.07
	Tat 30	AKANTSSASNKPISN		-	1.3	n.t.
	Vif 34	WTDVTPNYADILLHS			0.04	0.04
	Vif 36	TPNYADILLHSTYFP			0.06	0.08
83108	Gag 1	MGVRNSVLSGKKADE	-	0.14	n.t.	Dead
	Pol 25	VGFKLKQWPLSKEKIVALRE	0.07	0.09	1	Dead
	Env 8	VTESFDAWNNTVTEQAIEDV	0.03	-	n.t.	Dead
	Env 23	DKHYWDAIRFRYCAPPGYAL	0.06		-	Dead
	Env 59	LTVWGTKNLQTRVTAIEKYL	-		0.01	Dead
	Nef 66	RYPKTFGWLWKLVPV	-		0.02	Dead

[a] Sequences of peptides that induced positive IFN-γ production. Frequencies are given as percent IFN-γ staining in CD8^{+} or CD4^{+} lymphocytes (after subtraction of the background level).
[b] Acute infection: wk 2 or 3.
[c] Chronic infection: wk 12 or 13.
[d] Reactivities confirmed by in vitro stimulation are shaded gray. If minimal optimal has been described, it is bold and underlined within the 15/20-mer peptides.
[e] −, The peptide was tested, but the reactivity was equal to or below background.
[f] n.t., Peptide was not tested.
[g] Empty box, not tested because pool was negative.
[h]], Two peptides were pooled and tested together.
[i] If value is italic, then the value reported was from wk 2 or wk 12 instead of wk 3 and wk 13.

Table II. *Frequencies of CD4 responses in rhesus macaques after immunization and after challenge with SIVmac239*

Animal	Peptide	Sequence [a]	post 1. MVA	post 2. MVA	Acute[b] Infection	Chronic[c] Infection
97073	Gag 1	MGVRNSVLSGKKADE	0.02	g		
	Gag 82	IQNANPDCKLVLKGL [d]	0.01	-	-e	
	Nef 83	PWGEVLAWKFDPTLA	0.009	-	n.t.f	-h
	Nef 84	VLAWKFDPTLAYTYE	0.087	0.01	0.03i	
	Rev 18	RRQRKRRWRRRWQQL	0.12	0.01	-	-
	Rev 20	KRRWRRRWQQLLALA	0.09			
93062	Gag 46	GCTPYDINQMLNCVG	0.3		-	-
	Gag 47	YDINQMLNCVGDHQA	0.04		-	-
	Gag 61/62	TSSVDEQIQWMYRQQNPIP	-	-	0.14	-
	gag 63/64	QWMYRQQNPIPVGNIYRRW	-	-	0.13	-
	Gag 115	LMPTAPPEDPAVDLL	0.05		-	-
	Env 43	PKEQHKRNYVPCHIRQIINT	0.13	0.02	-	-
	Nef 66	RYPKTFGWLWKLVPV	0.07	-	-	-
	Nef 84	VLAWKFDPTLAYTYE	0.46	-	-	-
	Rev 4	ELRKRLRLIHLLHQT	0.35	-	┌─0.11	-
	Rev 6	RLRLIHLLHQTINPY	0.39	-		
	Rev 8	IHLLHQTINPYPTGP	0.08	-	n.t.	n.t.
	Rev 20	KRRWRRRWQQLLALA	0.15	-	0.04	-
	Rev 21	RRRWQQLLALADRIY	0.12	-	n.t.	n.t.
87081	Gag 14	NKEGCQKILSVLAPL				0.01
	Gag 15	CQKILSVLAPLVPTG				0.02
	Gag 39	RTLNAWVKLIEEKKF				0.01
	Gag 61	TSSVDEQIQWMYRQQ	-		0.05	-
	Gag 65	PIPVGNIYRRWIQLG	-		0.03	-
	Gag 66	GNIYRRWIQLGLQKC	-		0.09	-
	Gag 75	SYVDRFYKSLRAEQT			0.02	-
	Gag 76	RFYKSLRAEQTDAAV			0.05	-
	Gag 80	NWMTQTLLIQNANPD			0.03	-
	Gag 118	DLLKNYMQLGKQQRE	0.04		0.03	0.03
	Pol 29	NTPTFAIKKKDKNKWRMLID	0.02		-	-
	Env 36	KDAIKEVKQTIVKHPRYTGT	0.04		┌─0.01	0.03
	Env 37	IVKHPRYTGTNNTDKINLTA	0.03			0.01
	Nef 48	TMSYKLAIDMSHFIK	0.02		-	-
	Nef 65	GPGIRYPKTFGWLWK	0.07		┌─0.02	0.02
	Nef 66	RYPKTFGWLWKLVPV	0.2			0.01
	Rev 3	EREEELRKRLRLIHL	0.08		-	
	Rev 4	ELRKRLRLIHLLHQT	0.06		-	
	Rev 20	KRRWRRRWQQLLALA	0.12		0.04	-
	Rev 21	RRRWQQLLALADRIY	0.07		0.03	-
	Rev 27	PLDLAIQQLQNLAIE	0.02		-	n.t.
87082	Gag 25	HTEEAKQIVQRHLVV	0.3		-	n.t.
	Gag 26	AKQIVQRHLVVETGT	0.57		-	-
	Gag 49	CVGDHQAAMQIIRDI	0.14		-	-
	Nef 84	VLAWKFDPTLAYTYE	0.16		-	-
80035	Gag 1	MGVRNSVLSGKKADE	-	0.1	-	-
	Gag 8	YMLKHVVWAANELDR	0.016	-	-	┐-
	Gag 9	HVVWAANELDRFGLA	0.013	-	-	┘
	Gag 11	LDRFGLAESLLENKE			0.02	-
	Env 7	NGDYSEVALNVTESFDAWNN			0.02	n.t.	-
	Env 31/32	LNKYYNLTMKCRRPGNKTVLPVTIMSGLVF			-	0.045
	Env 35/36	QAWCWFGGKWKDAIKEVKQTIVKHPRYTGT			-	0.02
	Env 37/38	IVKHPRYTGTNNTDKINLTAPGGGDPEVTF			-	0.025
	Env 45/46	WHKVGKNVYLPPREGDLTCNSTVTSLIANI			-	0.035
	Nef 66	RYPKTFGWLWKLVPV	0.09	-	-	-
	Nef 84	VLAWKFDPTLAYTYE	0.02	-	n.t.	-
	Rev 3	EREEELRKRLRLIHL	0.03	0.01	┐-	┐-
	Rev 4	ELRKRLRLIHLLHQT	0.028	-	┘-	┘-
	Rev 18	RRQRKRRWRRRWQQL	0.01	-	┐-	┐-
	Rev 20	KRRWRRRWQQLLALA	0.023	-	┘	┘-
81035	Env 50	RLELGDYKLVEITPIGLAPT	0.4			-
	Nef 13	GETYGRLLGEVEDGY				0.06
	Nef 56	YSARRHRILDIYLEK				0.05
	Nef 58	RHRILDIYLEKEEGI				0.04
	Nef 84	VLAWKFDPTLAYTYE	0.1		-	-
96135	Gag 120	LGKQQREKQRESREK	0.04		-	-
	Gag 121	QREKQRESREKPYKE	0.02		-	-
	Env 35	QAWCWFGGKWKDAIKEVKQT	0.03		-	0.02
	Env 51	EITPIGLAPTDVKRYTTGGT	0.03		n.t.	n.t.
83108	Gag 1	MGVRNSVLSGKKADE	-	0.04	n.t.	Dead
	Gag 49	CVGDHQAAMQIIRDI	0.04	-	-	Dead
	Env 44	PCHIRQIINTWHKVGKNVYL	0.02		-	Dead
	Env 51	EITPIGLAPTDVKRYTTGGT	0.02		-	
	Nef 66	RYPKTFGWLWKLVPV	0.02		-	Dead
	Nef 83	PWGEVLAWKFDPTLA	0.07	-	n.t.	Dead
	Nef 84	VLAWKFDPTLAYTYE	0.11	-		Dead

[a] Sequences of peptides that induced positive IFN-γ production.
[b] Acute infection, wk 2 or 3.
[c] Chronic infection, wk 12 or 13.
[d] Reactivities confirmed by in vitro stimulation are shaded gray.
[e] −, The peptide was tested but the reactivity was equal to or below background.
[f] n.t., Peptide was not tested.
[g] Empty box, not tested because pool was negative.
[h]], Two peptides were pooled and tested together.
[i] If value is italic, then the value reported was from wk 2 or wk 12 instead of wk 3 and wk 13.

Differences between the cellular immune responses detectable in PBMC and lymphoid tissues

Epitope-specific T cell responses detected after vaccination in the periphery, but not during the chronic phase of infection, might still be present in lymphoid tissues. To investigate this possibility, we sacrificed animal 96135 at 40 wk postchallenge. This animal still had good CD4 counts in the peripheral blood at this time (>1000 CD4^{+} T cells/μl blood; Ref. 27). One week before sacrifice (wk 39), peripheral blood epitope-specific responses were analyzed by ICS of fresh PBMC. Consistent with previous findings, some epitope-specific responses detected in the periphery after vaccination were not detected at wk 39 postinfection (Table III, gray boxes). After necropsy, at 40 wk postchallenge, PBMC and mononuclear cells (MNC) from the spleen and axial, iliac, and mesenteric lymph nodes were isolated and then frozen. To account for the loss of APC after freeze/thawing, MNC were coincubated with autologous B-LCL at an MNC-B-LCL ratio of 10:1 during the 6.5 h ICS incubation time. This coincubation also eliminated the need for costimulatory Abs (Ref. 36 and our unpublished observation). As summarized in Table III, there were similarities and differences between the T cell responses detected in the peripheral blood and those detected in the lymphoid tissues. (Table III, gray boxes). With the exception of one response against Tat 15, all responses detected in the periphery at wk 39/40 were also detected in one or more of the lymphoid tissues. In contrast, most responses that were not detected in the periphery at wk 39 or 40 were still detected in at least one of the lymphoid tissues.

Viral escape does not account for the loss of certain peptide-specific responses after challenge

To investigate whether T cell responses against certain epitopes were lost because the virus accumulated mutations in these regions and therefore did not stimulate these T cell responses, whole virus was isolated from plasma of one monkey and directly sequenced. This method is able to detect regions of high variation within the virus (33). As shown in Fig. 3, the virus from the Mamu-A*01-positive animal 80035 accumulated mutations only in the Mamu-A*01-restricted Tat$_{28-35}$ SL8 epitope. This has been observed before in other Mamu-A*01-positive animals and does result in the escape of the virus from Tat$_{28-35}$ SL8-specific T cells (8). However, no other epitope accumulated mutations in the virus from this animal (Fig. 3), even though responses against some of them were not recalled after infection (e.g., Gag 1) or were lost after the acute phase (e.g., Nef 54).

Discussion

In this study, we mapped nearly the entire cellular immune response, mediated by CD8^{+} and CD4^{+} lymphocytes, in rhesus monkeys that were vaccinated against SIV and subsequently challenged with SIVmac239. Only a few studies have ever attempted to characterize the cellular immune response against all proteins expressed by the immunodeficiency viruses HIV or SIV at the level of epitopes recognized (39). Most studies thus far have only characterized the CD8^{+} T cell responses against a selection of epitopes. For this purpose, either a selection of specific tetramers relevant for the MHC class I alleles present in a given individual were used (8, 34, 40–46), or a selection of potential CTL epitopes in the form of peptides were used as stimulus in ICS assays or ELISPOT to identify responses (46, 47). Neither of these approaches provided a complete picture of the overall virus-specific CD8^{+} T cell responses. Even using a large selection of previously identified epitopes has its limitations, because each individual, with unique complement of MHC genes for each, may mount responses against previously unidentified epitopes (47). Many of the recent AIDS vaccine approaches aim to induce a good cellular immune response, which is believed to be crucial for the containment of the virus (1–8). It is therefore important to measure the

Table III. *Comparison of CD8 and CD4 responses in PBMC and lymphoid tissues from animal 96135 late in infection*

CD8 responses

Peptide	Sequence	week 12/13	PBMC week 39	week 40	Axial LN	Iliac LN	Mesenteric LN	Spleen
Gag 21	NTVCVIWCIHAEEKV	n.t.	-	n.t.	1.24ᵃ	1.16ᵃ	1.08ᵃ	1.35ᵃ
Gag 42	KKFGAEVVPGFQALS	-		-	n.t.			
Gag 45	ALSEG**CTPYDINQML**	n.t. — CM9	1.9	1.45	0.5	1.05	0.73	1.14
Gag 46	G**CTPYDINQML**NCVG	0.7						
Gag 67/68	RRWIQLGLQKCVRMYNPTN	n.t.	0.07	0.33	0.38		0.17	0.58
Gag 120/121	LGKQQREKQRESREKPYKE	-	-	n.t.	-	-	0.26	0.51
Pol 6	CYGQMPRQTGGFFRPWSMGK	-	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
Pol 40	VLEPPRKANPDVTLVQYMDD	-	-	n.t.	0.08	0.89	-	-
Pol 75	VNQIIEEMIKKSEIYVAWVP	0.1	-	n.t.	0.13	-	0.08	-
Env 10	WQLFETSIKPCVKLSPLCIT	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
Env 45	QAWCWFGGKWKDAIKEVKQT	-	-	-	0.05	1.3	-	0.96
Env 55	SAMGAASLTLTAQSRTLLAG	-	-	n.t.	-	-	-	-
Nef 31	YMNTPWRNPAEEREK	0.1	0.073	0.34	1.3	1.05	0.54	1.5
Nef 33/35	PWRNPAEEREKLAYR	-	0.11	n.t.	-	-	-	0.69
Nef 54	EKLAYRKQNMDDIDE	0.15	-	n.t.	-	-	-	0.67
Nef 49	KLAIDMSHFIKEKGG	0.058	-	n.t.	0.44	-	-	-
Nef 54	EGIYYSARRHRILDI	0.23	0.3	0.109	0.59	0.68	0.26	1.26
Nef 65	GPGIRYPKTFGWLWK	0.09	-	n.t.	1.2	1.17	0.58	-
Rev 4	ELRKRLRLIHLLHQT	0.07	-	n.t.	0.08	0.79	-	1.69
Tat 10	CISEADA**STPESANL**	n.t. — SL8	0.04	0.067	0.2	0.13	0.09	-
Tat 12	ADA**STPESANL**GEEI	0.26						
Tat 15	EEILSQLYRPLEACY	0.06	0.07	-	-	-	-	-
Tat 20	KKCCYHCQFCFLKKG	0.19	-	n.t.	0.18	0.11	0.01	-
Tat 21	YHCQFCFLKKGLGIC	0.07				0.1	0.16	0.02	-
Tat 30	AKANTSSASNKPISN	n.t.	-	n.t.	-	-	-	-
Vif 34	WTDVTPNYADILLHS	0.04	0.21	0.1	0.2	0.68	0.14	0.3
Vif 36	TPNYADILLHSTYFP	0.08						

CD4 responses

Peptide	Sequence	week 12/13	PBMC week 39	week 40	Axial LN	Iliac LN	Mesenteric LN	Spleen
Gag 120	LGKQQREKQRESREK	-	-	n.t.	0.8	-	0.033	-
Gag 121	QREKQRESREKPYKE	-						
Env 35	QAWCWFGGKWKDAIKEVKQT	0.02	0.12	0.006	0.017	-	0.045	0.24

ᵃ Differences between lymphoid tissues and PBMC and/or between PBMC samples from different time points are shaded gray (otherwise same footnotes as in Tables I and II).

entire cellular immune response against the immunodeficiency viruses to evaluate the efficacy of the experimental vaccine.

ICS facilitates the rapid identification of epitopes recognized in individuals without the knowledge of their MHC background. Recently, Goulder et al. (39, 48) have used a similar approach to identify all CD8⁺ responses in HIV-infected humans with the ELISPOT assay. However, data suggest that the ELISPOT assay is not as sensitive as ICS (20). We have previously shown that our ICS protocol is as sensitive as tetramer staining, at least in vaccinated animals or animals infected with pathogenic SIV <16 wk (34, 36). By using this ICS method, we were able to identify, in total, 92 CD8⁺ responses and 53 CD4⁺ responses against SIV proteins in 8 rhesus macaques. It is possible that some of the responses in different animals are directed against the same epitopes. The fine mapping and restriction analysis for all these responses are in progress. Only five of these CD8⁺ responses have been described before (Table I, Gag 45, Tat 12, Nef 63/64) and correspond to the two Mamu-A*01-restricted responses, $Gag_{181-189}$CM9 and Tat_{28-35}SL8 (8, 35) and the recently described Mamu-A*02-restricted $Nef_{159-167}$ YY9 epitope (49). The knowledge of these additional new CD8⁺ and CD4⁺ responses will greatly enhance our ability to study the interaction between the immune response and viral replication. This knowledge may also enhance the value of the rhesus monkey as model system for AIDS research.

This is the first study that investigated the evolution of the vaccine-induced T cell response in the peripheral blood after infection with SIV at the epitope level. Furthermore, we describe the continued evolution of the cellular immune response during infection and demonstrate that there was a difference between the T cell epitopes recognized after vaccination and after infection, at least with regard to the T cells detectable in the PBMC. Not all CD8⁺ responses and even fewer CD4⁺ responses detected after immunization were recalled after infection. In contrast, some of the vaccine-induced T-cells responses were recalled after virus infection and circulated through the blood. Additionally, new epitopes were detectable after infection with SIVmac239; many of these had not been observed after vaccination.

Our study raises a number of questions. Is it possible that the method of immunization resulted in the dominance of a different set of epitopes than those that dominate after infection with SIV? Each of the rMVA expresses only one or maximally two SIV proteins, and each rMVA was delivered to different sites in the skin. Therefore, competition at the level of Ag presentation, which can be a factor for immunodominance of certain epitopes over others (50), might be different during the vaccination in contrast to after SIV infection, when all proteins are expressed in the same APC. However, the DNA vaccine priming likely resulted in expression of most of the Ags in the same cell. The temporal sequence of viral gene expression can also influence immunodominance (50). Or is it possible that there could be a difference in the Ag processing of the proteins produced by the vaccine in contrast to proteins produced in SIV-infected cells? It has been shown previously that the production of epitopes can differ in different cell types (51). Vaccinia viruses have a very broad replication capability in different human cells (52), but because MVA is replication defective in human cells (53), the primary target cells after intradermal application might be skin fibroblasts (53) and to some degree skin dendritic cells (53, 54). In contrast, the immunodeficiency viruses replicate mostly in CD4⁺ cells (55–59). A difference in the cytokine environment during immunization in contrast to after SIV infection also could influence the Ag processing (60, 61). Infection with immunodeficiency viruses can result in an impairment of immune cells to produce IFN-γ (36, 62–66) and may also result in a change in the cytokine environment (67–70). The cytokine environment present during the induction of the immune response can impact the epitope specificity of the T cells induced (61). Indeed, the presence of IFN-γ can influence the specificity of proteasomes and alter the epitope-peptides being produced (60). These are important questions and need further investigation, because an effective vaccine would have to induce responses against epitopes that are produced by virus-infected cells.

An alternative explanation for the detection of new responses after infection could be that the vaccine primed for specific T cell responses that were below the limits of detection postvaccination but nevertheless were boosted to detectable levels after SIV infection. Previous studies have shown that animals with undetectable immune responses after DNA immunization show a robust response postinfection that significantly exceeds levels in animals that were not immunized, indicating that despite the lack of detectable responses postvaccination, the animals were primed (71–74). Another possibility is that the DNA vaccine may have primed for responses to specific epitopes that were not expanded by the MVA but were expanded by the SIV infection. Nevertheless, this does not explain the failure to detect vaccine-induced responses in the peripheral blood after infection.

Analysis of cellular immune responses in the present study was primarily limited to responses in the periphery. It is possible that some vaccine-induced responses that were undetectable in the peripheral blood after infection were still present in lymphoid tissues. To address this possibility, we compared immune responses present in the mucosal tissues with those present in the peripheral blood in a single animal. Our results indicate that certain epitope-specific T cells, which transiently circulate in the peripheral blood, may later reside predominantly in lymphoid tissues. Memory T-cells can acquire tissue-homing receptors (75, 76), which could
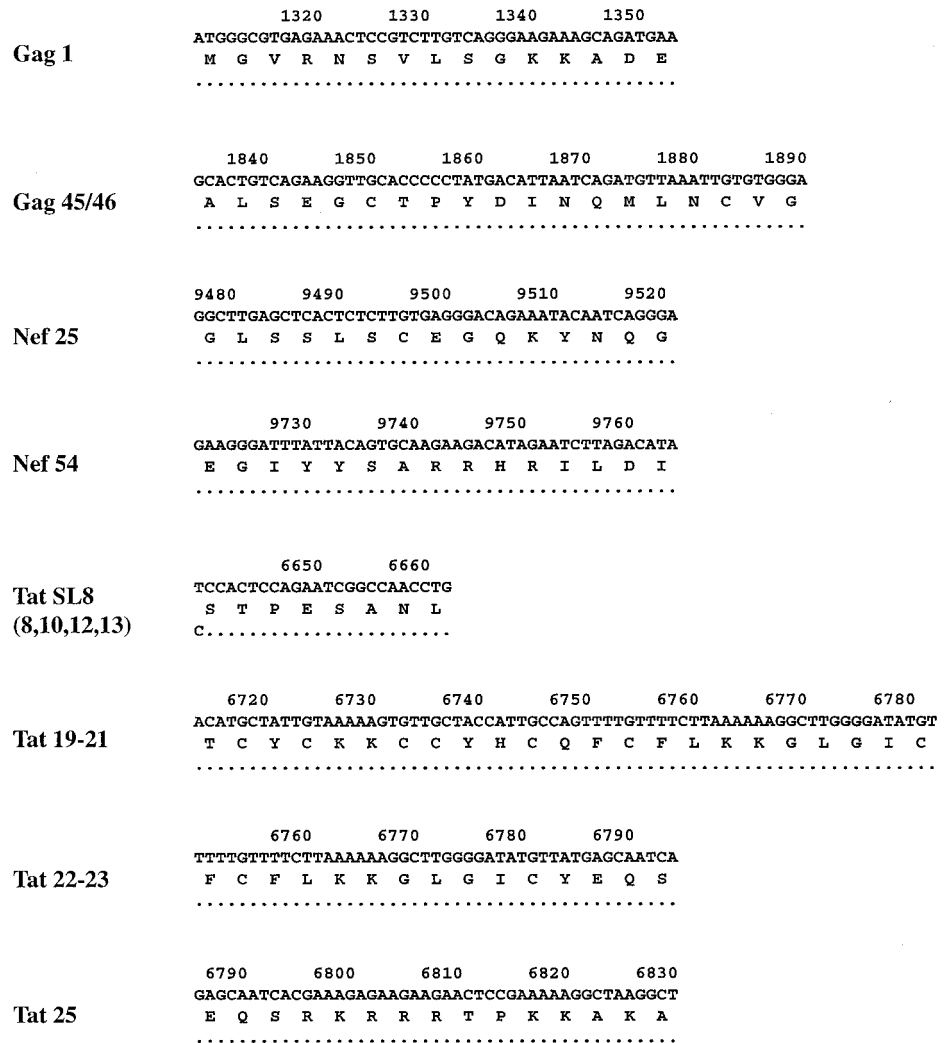
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                                         1320      1330      1340      1350
                                ATGGGCGTGAGAAACTCCGTCTTGTCAGGGAAGAAAGCAGATGAA
Gag 1                            M  G  V  R  N  S  V  L  S  G  K  K  A  D  E
                                .............................................


                                    1840      1850      1860      1870      1880      1890
                                GCACTGTCAGAAGGTTGCACCCCCTATGACATTAATCAGATGTTAAATTGTGTGGGA
Gag 45/46                        A  L  S  E  G  C  T  P  Y  D  I  N  Q  M  L  N  C  V  G
                                .........................................................


                                  9480      9490      9500      9510      9520
                                GGCTTGAGCTCACTCTCTTGTGAGGGACAGAAATACAATCAGGGA
Nef 25                           G  L  S  S  L  S  C  E  G  Q  K  Y  N  Q  G
                                .............................................


                                     9730      9740      9750      9760
                                GAAGGGATTTATTACAGTGCAAGAAGACATAGAATCTTAGACATA
Nef 54                           E  G  I  Y  Y  S  A  R  R  H  R  I  L  D  I
                                .............................................


                                       6650      6660
                                TCCACTCCAGAATCGGCCAACCTG
Tat SL8                          S  T  P  E  S  A  N  L
(8,10,12,13)                     C......................


                                  6720      6730      6740      6750      6760      6770      6780
                                ACATGCTATTGTAAAAAGTGTTGCTACCATTGCCAGTTTTGTTTTCTTAAAAAAGGCTTGGGGATATGT
Tat 19-21                        T  C  Y  C  K  K  C  C  Y  H  C  Q  F  C  F  L  K  K  G  L  G  I  C
                                .....................................................................


                                       6760      6770      6780      6790
                                TTTTGTTTTCTTAAAAAAGGCTTGGGGATATGTTATGAGCAATCA
Tat 22-23                        F  C  F  L  K  K  G  L  G  I  C  Y  E  Q  S
                                .............................................


                                  6790      6800      6810      6820      6830
                                GAGCAATCACGAAAGAGAAGAAGAACTCCGAAAAAGGCTAAGGCT
Tat 25                           E  Q  S  R  K  R  R  R  T  P  K  K  A  K  A
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FIGURE 3. Sequence analysis of virus from animal 80035 does not indicate viral escape in the majority of the epitopes. RT-PCR amplicons from viral cDNA were directly sequenced on an ABI 377 automated sequencer. The dominant viral sequence at each nucleotide site is shown; mixed bases with a secondary peak height of 50% of the maximal peak are indicated using standard International Union of Biochemistry nomenclature. With the exception of a mutation in the Tat_{28-35} SL8 epitope, which has been shown before to escape quickly in Mamu-A*01-positive animals (8), no mutations in other regions with CD8 epitopes were identified in this Mamu-A*01-positive animal.

explain this phenomenon. In addition, SIV predominantly replicates in mucosal tissues of the gastrointestinal tract (55, 77). Certain epitope specific T cells may therefore preferentially localize at these sites and transiently seed the periphery (78, 79). Results in the single animal tested in this study demonstrated a broader repertoire of T cell responses existed in the lymphoid tissue than the periphery at one time point. However, this study was not designed to address these issues. This part of the study was undertaken solely to establish whether epitope specificities that had been lost from the peripheral blood after infection could be detected in the lymphoid tissues. Nevertheless, our observation has implications for future vaccine studies. The measurement of the cellular immune response solely in the peripheral blood may not give a complete picture of all responses present in an individual. Further studies must investigate whether there is a qualitative difference between CD8 and CD4 responses present in the peripheral blood and in lymphoid or mucosal tissues to fully understand the potential of the cellular immune responses as defense mechanism against HIV infection. If T cells against certain epitopes, which predominantly reside in mucosal/lymphoid tissues, are more potent in containing the viral replication, then a vaccine should be directed at inducing these particular responses.

The disappearance of some $CD8^+$ responses during the chronic phase of SIV infection could be due to additional causes. Recent studies have demonstrated that HIV (5–7) and SIV (8, 9, 80) can accumulate mutations in CTL epitopes and that as a result the

responses against these epitopes can be lost (81, 82). Indeed, a study in HIV-infected patients also demonstrated the recognition of different epitopes in the chronic phase vs the acute phase of infection with HIV (48), similar to the results described in this study. We are currently exploring whether escape mutations may account for these findings by sequencing the entire virus at several time points after challenge. The limited data that we have at present suggests that viral escape does not account for the loss of some of the epitope specificities after infection (Fig. 3).

The disappearance of most virus-specific CD4 responses induced by vaccination shortly after infection could indicate that these activated cells were actually destroyed by the replicating virus, because SIV and HIV predominantly replicate in activated and/or memory $CD4^+$ cells (55, 57–59). A recent study by Douek et al. (83) has also demonstrated that HIV-specific $CD4^+$ T cells are preferentially infected. However, we were able to detect new CD4 responses in the acute and/or chronic phase of infection in some animals, indicating that these animals were still able to make SIV-specific responses, even in the presence of viral replication. It is possible that the reduced virus load in all these animals in comparison with that in the control animals after infection (27) prevented the complete destruction of the $CD4^+$ cells. However, there was no obvious correlation between the virus load in the acute or chronic phase of infection and the presence/absence of CD4 responses in these animals (27).

In conclusion, this study demonstrated that it is feasible to analyze the complete cellular immune responses against the immunodeficiency viruses using overlapping peptides spanning all proteins. Many new $CD8^+$ and $CD4^+$ T cell epitopes have been identified in this study, which will enhance the value of the rhesus macaque/SIV model for AIDS research and represents an important step toward the development and testing of a multiepitope AIDS vaccine in the SIV model. More importantly, the differences in the epitopes recognized after immunization and after infection in the peripheral blood could mean that measurement of immune responses in this compartment is not the best way to study vaccine-induced responses.

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