JOURNAL OF VIROLOGY, July 2006, p. 6801–6809 Vol. 80, No. 14
0022-538X/06/$08.00+0 doi:10.1128/JVI.00070-06

Preferential Infection Shortens the Life Span of Human Immunodeficiency Virus-Specific CD4$^+$ T Cells In Vivo

Jason M. Brenchley,[1] Laura E. Ruff,[1] Joseph P. Casazza,[2] Richard A. Koup,[2] David A. Price,[1] and Daniel C. Douek[1]*

Human Immunology Section[1] and Immunology Laboratory,[2] Vaccine Research Center, NIAID, NIH, Bethesda, Maryland

Received 10 January 2006/Accepted 1 April 2006

CD4$^+$ T-cell help is essential for effective immune responses to viruses. In human immunodeficiency virus (HIV) infection, CD4$^+$ T cells specific for HIV are infected by the virus at higher frequencies than other memory CD4$^+$ T cells. Here, we demonstrate that HIV-specific CD4$^+$ T cells are barely detectable in most infected individuals and that the corresponding CD4$^+$ T cells exhibit an immature phenotype compared to both cytomegalovirus (CMV)-specific CD4$^+$ T cells and other memory CD4$^+$ T cells. However, in two individuals, we observed a rare and diametrically opposed pattern in which HIV-specific CD4$^+$ T-cell populations of large magnitude exhibited a terminally differentiated immunophenotype; these cells were not preferentially infected in vivo. Clonotypic analysis revealed that the HIV-specific CD4$^+$ T cells from these individuals were cross-reactive with CMV. Thus, preferential infection can be circumvented in the presence of cross-reactive CD4$^+$ T cells driven to maturity by coinfecting viral antigens, and this physical proximity rather than activation status per se is an important determinant of preferential infection based on antigen specificity. These data demonstrate that preferential infection reduces the life span of HIV-specific CD4$^+$ T cells in vivo and thereby compromises the generation of effective immune responses to the virus itself; further, this central feature in the pathophysiology of HIV infection can be influenced by the cross-reactivity of responding CD4$^+$ T cells.

CD4$^+$ T helper cells are crucial for the maintenance of adaptive immunity. Specifically, CD4$^+$ T cells secrete cytokines that orchestrate subsequent immune responses and support homeostasis and CD4$^+$ T cells express costimulatory molecules that promote antigen presentation. In addition, CD4$^+$ T cells secrete cytokines and chemokines that have direct proinflammatory and antiviral properties. Indeed, the necessity of appropriate CD4$^+$ T-cell responses for control of viral replication is well documented (12, 19, 24). While human immunodeficiency virus (HIV) infection is relatively unique compared to other viral infections in that HIV is neither cleared nor controlled by the naturally elicited immune response, there are accumulating data suggesting that the HIV-specific CD4$^+$ T-cell response is critical in controlling viral replication. First, CD4$^+$ T cells that respond to HIV stimulation are found at higher frequencies in individuals classified as long-term nonprogressors (32). Second, individuals with detectable HIV-specific CD4$^+$ T-cell responses have a better prognosis after structured antiretroviral therapy interruptions (29, 33). Third, HIV-infected individuals that maintain HIV-specific CD4$^+$ T cells capable of producing interleukin-2 (IL-2) and proliferating have a better prognosis than individuals that do not have HIV-specific CD4$^+$ T cells capable of these effector functions (23, 44). Finally, HIV-specific CD4$^+$ T-cell responses are negatively correlated with disease progression (27). While HIV-specific CD4$^+$ T cells are paramount in control of viral replication, these cells are preferentially infected by the virus at all stages of infection (11, 14, 17).

The findings that HIV-specific CD4$^+$ T cells are preferentially infected by the virus and that infected CD4$^+$ T cells are less likely to reach terminal differentiation in vivo (5) led us to question whether or not preferential infection impacts the ability of HIV-specific CD4$^+$ T cells to undergo rounds of stimulation and proliferation required to increase precursor frequencies to appreciable levels and reach terminal differentiation in vivo. Indeed, earlier reports suggest functional and phenotypic abnormalities within the HIV-specific CD4$^+$ T-cell pool. However, the causality of this phenomenon is unclear. Here, we attempt to address this issue by a detailed study of phenotype and magnitude of HIV-specific CD4$^+$ T cells compared to those of cytomegalovirus (CMV)-specific CD4$^+$ T cells in a cohort of HIV-infected individuals; in doing so, we relate observed interindividual differences to infection rates within CD4$^+$ T cells of defined antigen specificity. In particular, a detailed analysis of uncommon and atypical responses provided important clues to mechanisms underlying the ability to circumvent preferential infection and provided insight into vaccine modalities aimed at stimulating HIV-specific CD4$^+$ T cells.

MATERIALS AND METHODS

Subjects. Twenty-three HIV- and CMV-dually infected and 11 CMV-infected subjects were recruited at the National Institutes of Health (Table 1). Plasma HIV load was determined using either a Roche Amplicor Monitor assay or a Roche Ultradirect assay. Informed consent was obtained from all subjects prior to entry into this study, which was approved by the NIH Institutional Review Board.

Cell stimulation. Peripheral blood mononuclear cells (PBMC) were isolated and in some instances viably cryopreserved until later use. Antigenic stimulation was performed with fresh or frozen PBMC as previously described (2, 28). Similar results from cell stimulation protocols were obtained using fresh or frozen PBMC (7). In every experiment, a negative control (anti-CD28/CD49d, 1 μg/ml final) was included to quantify spontaneous production of effector cytokines, and a positive control (staphylococcus enterotoxin B, 1 μg/ml final; Sigma, St. Louis, MO) was included to ensure that cells were responsive to in vitro stimulation. In all experiments, less than 0.1% of CD4$^+$ T cells produced effector cytokine in the absence of specific stimulation. Overlapping peptides were used

* Corresponding author. Mailing address: VRC, NIH, Bldg. 40, Rm. 3509, 40 Convent Drive, Bethesda, MD 20892. Phone: (301) 594-8484. Fax: (301) 480-2565. E-mail: ddouek@mail.nih.gov.

TABLE 1. Subject cohort

Subject no.	HIV status[a]	CD4 count	Virus load	HAART
1	+	304	200,000	No
2	+	459	129	No
3	+	157	61,400	No
4	+	394	57,200	No
5	+	491	6,400	No
6	+	939	3,900	No
7	+	636	<50	Yes
8	+	471	800	Yes
9	+	636	2,500	No
10	+	884	<50	Yes
11	+	327	125	Yes
12	+	297	5,600	No
13	+	145	3,200	Yes
14	+	100	185,200	No
15	+	552	21,900	No
16	+	602	12,500	No
17	+	343	200,000	No
18	+	316	376,200	No
19	+	400	71,000	No
20	+	708	<50	Yes
21	+	1,058	2,600	No
22	+	652	31,400	No
23	+	469	7,500	No
24–34	−	NA[b]	0	No

[a] +, positive; −, negative.
[b] NA, not applicable.

to stimulate HIV-specific T cells in the presence of brefeldin A (1 μg/ml; Sigma) for 5 h at 37°C. All cells were surface stained for phenotypic markers of interest and intracellularly stained for cytokines.

Antigens. Fifteen-mer peptides overlapping by 11 amino acids corresponding to sequences of a clade B HXBc2/Bal R5 chimeric HIV strain (Gag, Pol, Env, and Nef) were pooled into mixtures grouped by HIV proteins as previously described (2). Individual peptides were suspended in dimethyl sulfoxide at 10 mg/ml and diluted to a final concentration of 2 μg/ml in all stimulations. CMV whole antigen and control antigen complement fixation lysate preparations were obtained from BioWhittaker (Walkersville, Maryland) and were used at 60 μl/ml for stimulations.

Immunofluorescence staining. Stimulated PBMC were washed and then stained with directly conjugated antibodies to surface markers (Becton Dickinson Immunocytometry Systems [BDIS], San Jose, CA) for 20 min on ice. The cells were washed and fixed/permeabilized for 10 min (fixation/permeabilization solution; BDIS), stained with directly conjugated antibodies to gamma interferon (IFN-γ), tumor necrosis factor (TNF), and IL-2 all conjugated to the same fluorochrome, and resuspended in 1% paraformaldehyde in phosphate-buffered saline. The use of monoclonal antibodies against three effector cytokines simultaneously increases the probability of identifying relevant antigen-responsive $CD4^+$ T cells (28). Responses were considered to be antigen specific if responding cells represented greater than 0.1% of all $CD4^+$ T cells.

MHC-II tetramers. HLA DR 15*01 soluble tetramers loaded with either HIV p24-4 (DRFYKTLRAEQASQ) or CLIP (PVSKMRMATPLLMQA) were prepared as previously described and were a kind gift from Kai Wucherpfennig (9, 38). Major histocompatibility complex class II (MHC-II) multimers were then added to at least one million lymphocytes at 10 μg/ml at 37°C for 20 min. Cells were then washed, stained with surface markers of interest, and analyzed immediately.

Flow cytometric analysis. Six-parameter flow cytometric analysis was performed using a FACSCalibur flow cytometer (BDIS). Fluorescein isothiocyanate (FITC), phycoerythrin (PE), peridinin chlorophyll protein, and allophycocyanin (APC) were used as the fluorophores. At least 100,000 live $CD3^+$ lymphocytes were collected. Eight-parameter flow cytometric sorting and analysis were performed using a FACS Aria flow cytometric cell sorter (BDIS). FITC, PE, APC, Cascade Blue, and Cy7 APC were used as the fluorophores. The list mode data files were analyzed using FlowJo software (Tree Star, Inc., San Carlos, CA).

Cell sorting. Cell sorting was accomplished using a FACS Aria cell sorter (BDIS) at 70 lb/in^2. FITC, PE, Cy5 PE, and APC were used as the fluorophores. At least 10,000 cells were sorted for PCR and reverse transcriptase PCR analysis. Sorted populations were consistently at least 99.8% pure.

qPCR. Quantification of HIV *gag* DNA in sorted $CD4^+$ T cells was performed by quantitative PCR (qPCR) by means of a 5′ nuclease (TaqMan) assay with an ABI7700 system (PerkinElmer, Norwalk, CT) as previously described (5, 13). To quantify cell number in each reaction, qPCR was performed simultaneously for albumin gene copy number as previously described (14). Standards were constructed for absolute quantification of *gag* and albumin copy number and were validated with sequential dilutions of 8E5 cell lysates that contain one copy of *gag* per cell. Duplicate reactions were run and template copies calculated using ABI7700 software.

Clonotypic analysis. Viable antigen-specific $CD4^+$ T cells were sorted based upon capture of IFN-γ following antigen-specific stimulation with a CD45/IFN-γ bispecific antibody as described previously (13). Clonotypic analysis was performed as described previously by using a modified template switch anchored reverse transcriptase PCR with a 3′ constant region primer to amplify all expressed T-cell receptor β-chain (TCRB) gene products without bias (13, 31).

Statistical analysis. Statistical significance was determined by a Mann-Whitney test using Prism 3.0 software (Prism, San Diego, CA).

RESULTS

Small frequencies of circulating $CD4^+$ T cells are HIV specific. Studies suggest that HIV-infected individuals generally do not maintain large populations of HIV-specific $CD4^+$ T cells (28). This is counterintuitive because one would expect that under conditions of chronic viral replication a high frequency of antigen-specific $CD4^+$ T cells should be expanded in the memory T-cell pool (14, 17, 28, 44). To explore these observations further, we initially used intracellular cytokine staining following antigenic stimulation with HIV Gag, Nef, Env, or Pol or CMV whole antigen. Consistent with previous studies, we found that significantly fewer $CD4^+$ T cells responded to HIV-specific stimulation than to CMV-specific stimulation (Fig. 1). Here, individual responses to HIV Gag, Nef, Env, and Pol are summed, as these proteins represent the

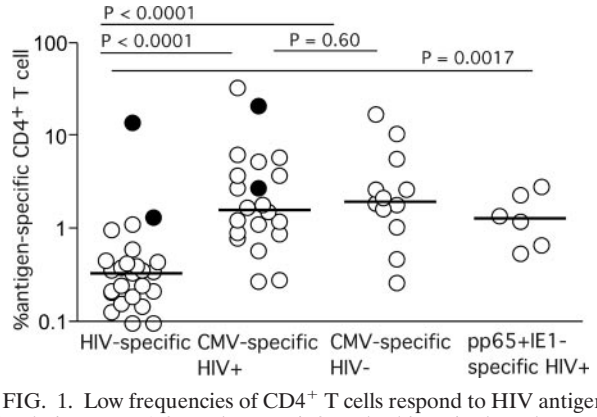

FIG. 1. Low frequencies of $CD4^+$ T cells respond to HIV antigenic stimulation. PBMC from the HIV-infected subjects in the cohort were stimulated with peptides 15 amino acids in length and overlapping by 11 amino acids and grouped according to antigen. Peptides encompassing HIV Gag, Env, Pol, and Nef were used as stimulants. PBMC from all individuals were also stimulated with CMV whole antigen. In addition, for six individuals, CMV responses after stimulation with overlapping peptides encompassing CMV pp65 and IE1 are included for completeness. Following stimulation for 5 h in the presence of brefeldin A, cells were extracellularly stained for CD4, CD3, and CD57 and were intracellularly stained for TNF, IL-2, and IFN-γ. The percentages of $CD4^+$ T cells that responded with cytokine secretion are displayed. HIV-specific $CD4^+$ T-cell responses represent summation of responses to HIV Gag, Nef, Env, and Pol. Filled symbols represent results for subjects 1 and 21. Horizontal bars represent medians.

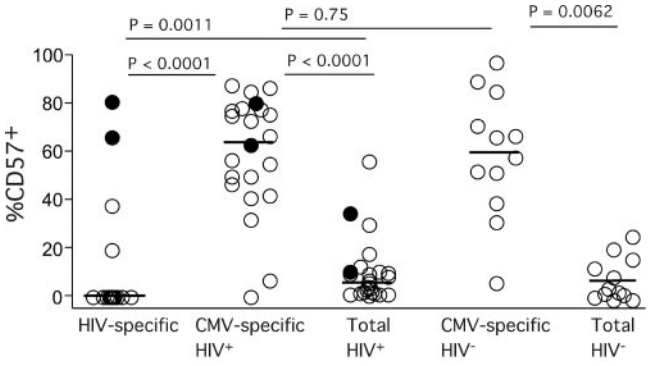

FIG. 2. CD4+ T cells that respond to HIV-specific stimulation do not reach terminal differentiation in vivo. PBMC from individuals in the cohort were stimulated with HIV peptides and/or CMV whole antigen. The percentage of responding antigen-specific CD4+ T cells that reached terminal differentiation was determined based upon expression of CD57. HIV-specific CD4+ T-cell responses represent summation of responses to HIV Gag, Nef, Env, and Pol. Filled symbols represent results for subjects 1 and 21. Horizontal bars represent medians.

most immunogenic HIV proteins during chronic HIV infection (1). Importantly, the frequency of responding HIV-specific CD4+ T cells in HIV-infected individuals was also significantly smaller than the frequency of responding CMV-specific CD4+ T cells in HIV− CMV+ individuals, even though both cellular and free viral loads were higher for HIV than for CMV (Fig. 1). Furthermore, there was no statistical difference between the frequencies of CD4+ T cells that respond to CMV when comparing the HIV-infected individuals to the HIV-uninfected individuals. However, CMV stimulation using whole antigen lysate preparations is not necessarily directly comparable to HIV-specific stimulations based upon overlapping peptide stimulations. We therefore confirmed that significantly lower frequencies of CD4+ T cells respond to HIV stimulation than to CMV stimulation by stimulating CD4+ T cells from several CMV-seropositive individuals with 15-mer overlapping peptides encompassing CMV pp65 and IE1. While these proteins are immunostimulatory in many individuals, this stimulation protocol invariably results in an underrepresentation of the total CMV-specific CD4+ T-cell population (40). Nonetheless, the frequency of CD4+ T cells that respond to these two proteins of CMV is significantly higher than the frequency of CD4+ T cells that respond to HIV Gag, Env, Nef, and Pol (Fig. 1).

Functionally defined HIV-specific CD4+ T cells are not terminally differentiated. Under normal circumstances, chronic viral replication should result in clonal expansion of antigen-specific CD4+ T cells, as is the case for HIV-specific CD8+ T cells (6). While the ability of CD4+ T cells to reach terminal differentiation is substantially different from that of CD8+ T cells (36, 43), it is generally accepted that terminally differentiated CD4+ T cells accumulate in situations of chronic viral infections and in the chronic stages of HIV infection (5, 17, 22). We measured surface CD57 expression to determine whether or not HIV-specific CD4+ T cells became terminally differentiated in vivo compared to CMV-specific CD4+ T cells and other memory CD4+ T cells (Fig. 2). HIV-specific CD4+ T cells reach terminal differentiation at a significantly lower frequency than CMV-specific (HIV-infected or HIV-uninfected) and other memory (HIV-infected) CD4+ T cells, demonstrating that terminally differentiated CD4+ T cells that accumulate in the peripheral blood of HIV-infected individuals do not respond to the virus itself. Importantly, there was no difference between the frequencies of CMV-specific CD4+ T cells that reached terminal differentiation when comparing HIV-infected and uninfected individuals (Fig. 2).

Physically defined HIV-specific CD4+ T cells are not terminally differentiated. Identification of antigen-specific CD4+ T cells from humans usually involves a functional readout after antigen-specific stimulation. However, the recent advent of soluble MHC-II multimers has allowed for physical identification of antigen-specific CD4+ T cells (9, 18, 35, 38). To ensure that our analysis was not biased to detection of only HIV-specific CD4+ T cells capable of effector function, we utilized HLA DR 15*01 tetramers to identify antigen-specific CD4+ T cells from subject 9 (known to express the HLA DR 15*01 allele and respond to the p24-4 peptide) (Fig. 3). Lymphocytes were stained with tetramers binding either Gag peptide or CLIP peptide as a negative control and then stained for surface molecules of interest. For this individual, no CD4+ T cells specific for p24-4 became terminally differentiated in vivo. We were unable to identify HIV-specific CD4+ T cells with HLA DR 15*01 tetramers for any other individuals in our cohort due to limiting frequencies of responses to individual peptides and lack of appropriate HLA haplotypes.

HIV-specific CD4+ T-cell responses can be large and composed of phenotypically mature T cells when preferential infection is avoided. We further explored the impact of preferential infection by close examination of the magnitude and corresponding phenotypes of the responding HIV-specific CD4+ T cells from the subjects in our cohort and observed that two subjects were clearly outliers (Fig. 1 and 2). For these two individuals (subjects 1 and 21), the CD4+ T cells that responded to HIV antigens were of phenotype and magnitude similar to those of CD4+ T cells that responded to CMV antigens (Fig. 4). In order to understand the unusual circumstances that allowed these two individuals to respond to HIV-specific stimulation with such a large frequency of terminally differentiated CD4+ T cells, we sorted subsets of CD4+ T cells from these individuals (Fig. 4) and performed qPCR for HIV DNA, which acts as a surrogate for in vivo infection history (5, 14) (Table 2). The responding HIV-specific CD4+ T cells sorted from these two individuals were infected at frequencies similar to those of other memory CD4+ T cells, demonstrating that the HIV-specific CD4+ T cells of these two subjects were not preferentially infected by HIV. In contrast, and consistent with our previous studies and those of others (11, 14, 17), the HIV-specific CD4+ T cells from subject 19 were preferentially infected (Table 2).

Clonotypic analysis of CMV- and HIV-specific CD4+ T cells. Although subjects 1 and 21 both had high frequencies of terminally differentiated, HIV-specific CD4+ T cells, both subjects failed to control viral replication. Moreover, subject 1 progressed to AIDS (Table 1). In any event, the ability of these two individuals to respond to HIV-specific stimulation with a high frequency of CD4+ T cells in the absence of preferential infection presented an opportunity to dissect the mechanistic basis for these anomalous responses.

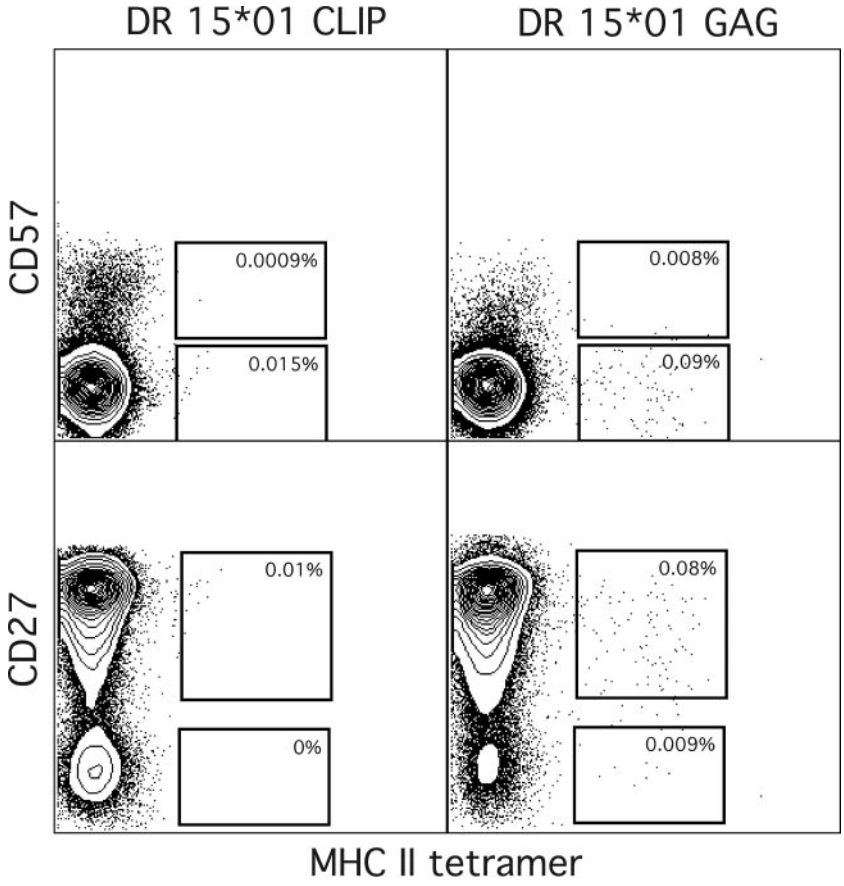

FIG. 3. Physically identified HIV-specific $CD4^+$ T cells do not reach terminal differentiation in vivo. PBMC from subject 9 were surface stained with soluble HLA DR 15*01 tetramers bound to either irrelevant peptide or HIV p24 DRFYKTLRAEQASQ. PBMC were then washed and surface stained with anti-CD3, anti-CD4, anti-CD27, and anti-CD57. $CD4^+$ $CD3^+$ events are shown.

To characterize further the nature of these $CD4^+$ T cells, we first used epitope mapping based on matrix analysis (2) to determine that subject 1 responded to a single 15-mer peptide in HIV Gag p24 (EQIGWMTNNPPIPVG). We then used this single HIV 15-mer peptide to stimulate HIV-specific $CD4^+$ T cells from subject 1. In addition, in a parallel experiment we stimulated CMV-specific $CD4^+$ T cells from the same individual. For both antigen-specific responses, we viably sorted the responding T cells by utilization of an antibody bispecific for IFN-γ and CD45 (13). Cells that captured IFN-γ and expressed CD69 were then separated and sorted based on CD57 expression. We then determined the clonotypic composition of the responding CMV- and HIV-specific $CD4^+$ T cells by sequencing the CDR3s of all expressed TCRB genes (Fig. 5) as previously described (13, 31). In addition, in a separate experiment we sorted $CD57^+$ $CD4^+$ T cells from this individual without regard to their antigen specificity and performed clonotypic analysis (Fig. 5A).

From this analysis, we were surprised to find that the HIV-specific $CD4^+$ T cells from subject 1 were clearly cross-reactive with CMV. Importantly, the bulk $CD57^+$ $CD4^+$ T-cell subset from this individual was composed of two dominant clonotypes (Fig. 5A). The CMV-specific $CD4^+$ T cells were also dominated by these same two clonotypes, suggesting that all $CD57^+$ $CD4^+$ T cells from this individual were CMV specific (Fig. 5B). One of these predominant clonotypes also responded to HIV-specific stimulation (Fig. 5C). This dominant clonotype found within HIV- and CMV-specific $CD57^+$ $CD4^+$ T cells was also found in both HIV- and CMV-specific $CD57^-$ $CD4^+$ T cells (Fig. 5D and E). In order to confirm that this dominant clonotype was expressed by a cross-reactive T-cell clone (and not two different T-cell clones expressing the same TCRBV), we also sequenced the TCRA region and found exactly the same sequences between T cells sorted after stimulation with CMV or HIV (data not shown). We found no sequence homologies between the HIV epitope and the CMV proteome and were therefore unable to identify the individual CMV epitope. However, cross-reactivity can exist among epitopes that share no primary structure similarities (42). While we were unable to determine the relative affinities that these cross-reactive T cells have for the CMV and HIV epitopes, we were able to determine that the T cells could respond to the HIV epitope at concentrations as low as 0.0125 μg/ml (data not shown).

In addition, we performed epitope mapping from subject 21 (the other individual that was able to maintain HIV-specific $CD4^+$ T cells that avoided preferential infection). In stark contrast to subject 1, subject 21 responded to at least six epitopes within HIV Gag (data not shown). Unfortunately, no response was large enough to facilitate use of viable sorting in order to perform clonotypic analysis. We therefore stimulated

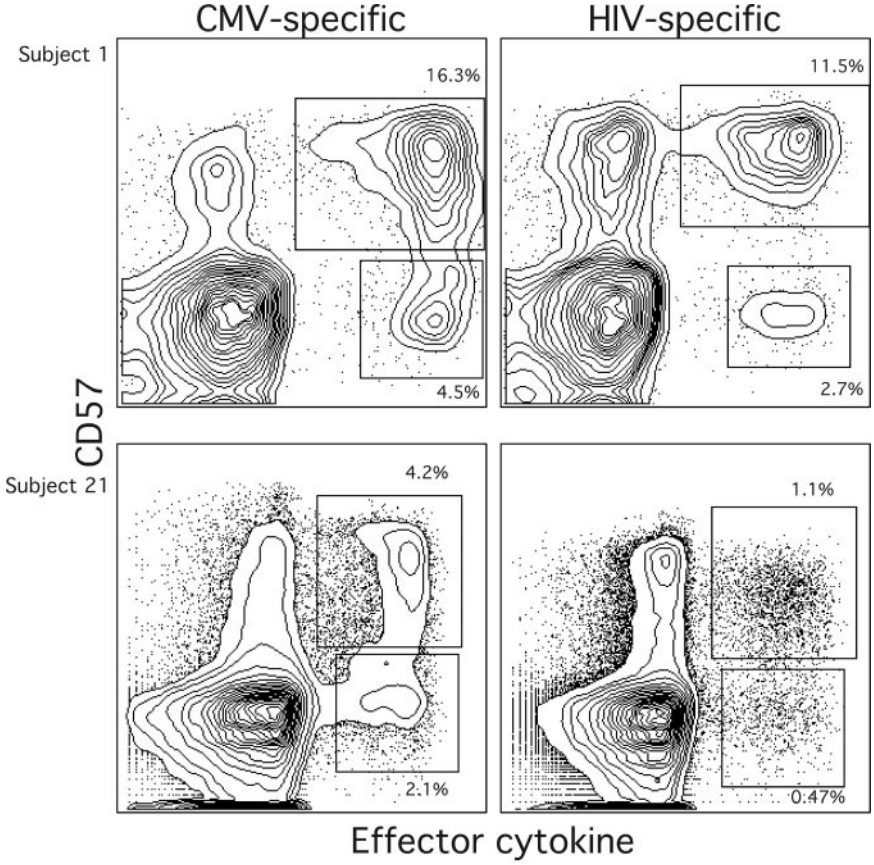

FIG. 4. HIV-specific CD4+ T cells can be present at high frequencies and composed of terminally differentiated CD4+ T cells. PBMC from subjects 1 and 21 were stimulated with Gag peptides or CMV whole antigen. Following stimulation for 5 h in the presence of brefeldin A, cells were extracellularly stained with CD4, CD3, and CD57 and were intracellularly stained for TNF, IL-2, and IFN-γ. CD4+ CD3+ events are shown.

CD4+ T cells with peptides encompassing all of Gag for viable sorting of antigen-specific CD4+ T cells followed by clonotypic analysis (Fig. 6). In a parallel experiment, we sorted CD4+ T cells that responded to CMV-specific stimulation followed by clonotypic analysis (Fig. 6). While the CMV-specific CD4+ T cells from this individual were oligoclonal, dominated by three clonotypes, the HIV-specific CD4+ T cells were extremely polyclonal, and of this polyclonal repertoire one clone was cross-reactive with CMV.

TABLE 2. Cell-associated viral DNA

Subject no.	T-cell subset	Frequency of viral DNA/10^5 sorted cells (% infected)
1	CD57− memory	1,388 (1.39)
	CD57+ memory	209 (0.2)
	CD57− HIV specific	248 (0.25)
	CD57+ HIV specific	54 (0.05)
	CD57− CMV specific	575 (0.58)
	CD57+ CMV specific	114 (0.11)
21	CD57− memory	580 (0.58)
	CD57+ memory	254 (0.25)
	CD57− HIV specific	705 (0.7)
	CD57+ HIV specific	388 (0.39)
19	CD57− memory	779 (0.8)
	CD57+ memory	82 (0.082)
	CD57− HIV specific	2,337 (2.3)

DISCUSSION

In the present study, we have examined the magnitudes, maturational phenotypes, clonotypic structures, and HIV infection frequencies of CD4+ T-cell populations that respond to HIV-derived antigens. The principal findings were as follows: (i) in the majority of HIV-infected individuals, the frequency of HIV-specific CD4+ T cells is small and the responding cells do not reach terminal differentiation in vivo despite chronic exposure to high levels of antigen; (ii) in a rare minority of HIV-infected individuals, high frequencies of terminally differentiated HIV-specific CD4+ T cells can be detected; (iii) these large, phenotypically mature populations of HIV-specific CD4+ T cells are not preferentially infected by HIV; and (iv) this lack of preferential infection is associated with HIV-specific CD4+ T-cell clonotype cross-reactivity with CMV. Taken together, these data indicate that preferential infection adversely affects the HIV-specific CD4+ T-cell response to a substantial extent and provide evidence that proximity of HIV-specific CD4+ T cells to antigen-presenting cells that present HIV-derived epitopes and perhaps infectious HIV virions during antigen presentation in vivo is involved in this process.

A. Total CD57$^+$ CD4$^+$ T cells

TCRBV	CDR3	TCRBJ	%freq
12-4	CASRSPAYNEQF	2.1	47
12-3	CASSPLDNQPQH	1.5	28
6-5	CASSSRPTGSNQPQH	1.5	12
5-1	CASSLGGVRDNEQF	2.1	2
5-1	CASSGESSYEQY	2.7	2
12-3	CANSPLDNQPQH	1.5	2
12-3	CVSSPLDNQPQH	1.5	2
12-3	CAGSPLDNQPQH	1.5	2
12-4	CASSPPRDSKETRY	2.5	2
12-4	CASSPPRDGKETQY	2.5	2
20-1	CSALRLAGGPGEQY	2.7	2

B. CMV-specific CD57$^+$ CD4$^+$ T cells

TCRBV	CDR3	TCRBJ	%freq
12-4	CASRSPAYNEQF	2.1	45
12-3	CASSPLDNQPQH	1.5	32
4-1	CASRPEDDWRNTIY	1.3	9
19	CASSIQVTNEKLL	1.4	3
5-1	CASSLGGSAEAF	1.1	2
5-1	CASSFSGGEQF	2.1	2
5-1	CASSLGGVRDNEQF	2.1	2
6-5	CASSSRPTGSNQPQH	1.5	2
12-3	CAGSPLDNQPQH	1.5	2
12-3	CASRSPAYNEQF	2.1	2
12-4	CASRSPACNEQF	2.1	2

C. HIV-specific CD57$^+$ CD4$^+$ T cells

TCRBV	CDR3	TCRBJ	%freq
12-4	CASRSPAYNEQF	2.1	54
9	CASSARLAGAELF	2.2	6
3-1	CASSQMLGSNEQF	2.1	4
5-1	CASSSFGGFSYNEQF	2.1	4
7-9	CASSLGLPGPAGYT	1.2	4
9	CASSVEARGRTGELF	2.2	2
18	CASSPGSYGYT	1.2	2
27	CASRRAGQPSSGNTIY	1.3	2
28	CASMTGADTDTQY	2.1	2
3-1	CASSEEGTSWSSYEQY	2.7	2
5-5	CASSSLGLSNQPQH	1.5	2
6-6	CASSYSVQGGGNTEAF	1.1	2
7-9	CASSYINTGELF	2.2	2
7-9	CASSLEAIQVGANVLT	2.6	2
12-3	CASSPLDNQPQH	1.5	2
12-3	CASRSPAYNEQF	2.1	2
20-1	CSARDQGKYGYT	1.2	2
20-1	CSASGPYTGELF	2.2	2
20-1	CSAIGQTYEQY	2.7	2

D. CMV-specific CD57$^-$ CD4$^+$ T cells

TCRBV	CDR3	TCRBJ	%freq
12-4	CASRSPAYNEQF	2.1	33
12-3	CASSPLDNQPQH	1.5	11
27	CASSLFGGESENIQY	2.4	4
3-1	CASSPRDREGEKLF	1.4	4
6-5	CASSYSRSSGSLDTQY	2.3	4
20-1	CSARGRLGVNTGELF	2.2	4
19	CASSMGGATSEQF	2.1	3
4-2	CASSQDNAQLRTDTQY	2.3	3
5-1	CASSLRMGPDKNTGELF	2.2	3
6-1	CASSERGAASPDTQY	2.3	3
7-2	CASSLQTGIGTDTQY	2.3	3
3-1	CASSSRARQETQY	2.5	1
4-1	CASSQGASSNTGELF	2.2	1
5-1	CASSVGGLKGI	2.3	1
6-5	CASSSRPTGSNQPQH	1.5	1
6-5	CASSYSGTAYNSPLH	1.6	1
7-2	CASSPGLVATDTQY	2.3	1
7-2	CASSSGQGGGKTQY	2.5	1
7-2	CASSSVPGLY	2.5	1
7-3	CASSSVPGLY	2.5	1
7-8	CASSRTGDVSNQPQH	1.5	1
7-9	CASSLRGTRATNEKLF	1.4	1
7-9	CASSLDSHWDDEQF	2.1	1
7-9	CASSRWGSAVKETQY	2.5	1
10-3	CAISESAPNTGELF	2.2	1
12-3	CASGPGGGTQPQH	1.5	1
12-4	CASRSPAYNGQF	2.1	1
20-1	CSASRGTSGNQETQY	2.5	1
20-1	CSVETSGRSYEQY	2.7	1
24-1	CATSDGTRETQY	2.5	1

E. HIV-specific CD57$^-$ CD4$^+$ T cells

TCRBV	CDR3	TCRBJ	%freq
12-4	CASRSPAYNEQF	2.1	42
28	CASMMYPEQF	2.1	8
6-6	CASSIQRATEAF	1.1	8
28	CASDGGRDYEQY	2.7	7
10-3	CAISESRTLGSQPQH	1.5	6
18	CASSVQGATTEAF	1.1	3
5-1	CASSSGTGNLKGLYEQY	2.7	3
7-2	CASSLGQGRSGYT	1.2	3
2	CASSSTSVGAGELF	2.2	1
15	CATSREGRGRGNEKLF	1.4	1
18	CASSPRVYGQEDYEQY	2.7	1
19	CASSIASGTGWTGANVLT	2.6	1
27	CASSSWTGVGNTEAF	1.1	1
27	CASSAPLLSGVGGLGTQY	2.5	1
28	CASSPSWDQPQH	1.5	1
3-1	CASSQVSGGAYEQY	2.7	1
5-1	CASSGNYGAGELF	2.2	1
6-5	CASSITGDTEAF	1.1	1
6-5	CASSSGQGYSEQY	2.7	1
7-6	CASSLTGSNTEAF	1.1	1
10-3	CAISEPRTLGSQPQH	1.5	1
12-4	CAGRSPAYNEQF	2.1	1
29-1	CSVILGSSGYEQY	2.7	1

FIG. 5. Clonotypic analysis of HIV- and CMV-specific CD4$^+$ T cells from subject 1. PBMC from subject 1 were stimulated with Gag EQIGWMTNNPPIPVG or CMV whole antigen in the presence of a CD45/IFN-γ bispecific antibody. Following stimulation for 5 h, cells were extracellularly stained with CD4, CD3, CD57, and IFN-γ. IFN-γ-producing cells were sorted based upon CD57 expression into RNAlater followed by unbiased anchored template switch PCR to identify all expressed TCRB genes. freq, frequency.

Historically, CD4$^+$ T-cell responses to HIV-derived antigens have been difficult to detect in infected individuals. While early attempts were potentially limited by methodological constraints (8, 41), more sensitive techniques that can be applied directly ex vivo have generally confirmed these initial observations (28). However, it is also clear that, under certain circumstances, HIV-specific CD4$^+$ T-cell responses of substantial magnitude can be present in the peripheral blood of infected individuals. For example, during primary HIV infection, vigorous CD4$^+$ T-cell responses to HIV-derived antigens can be detected frequently (26, 32); these can be transient in nature or more sustained and tend to be preserved in the short term by the early administration of highly active antiretroviral therapy (HAART) (20, 26, 33). Similarly, substantial HIV-specific CD4$^+$ T-cell responses have been observed with long-term nonprogressors (3) and with individuals with "discordant" responses to HAART, in whom virologic breakthrough is associated with drug-resistant HIV typically exhibiting impaired

A. CMV-specific CD4+ T cells

TCRBV	CDR3	TCRBJ	%freq
18	CASSPTSGDTQY	2.3	38
19	CASSSSPTGNIQY	2.4	17
2	CASSEAAQAPLH	1.6	16
20-1	CSAPTGKGDEQF	2.1	4
2	CASTYTDTQY	2.3	3
2	CAASQQGAQDTQY	2.3	3
5-1	CASSLAEPTYNEQF	2.1	3
18	CASSPISTDTQY	2.3	3
20-1	CSAKVGKEGGYT	1.2	3
5-1	CASSFDPGTGGREQF	2.1	1
5-4	CASSAGQGRTEAF	1.1	1
7-2	CASSLWGDSGNTIY	1.3	1
7-2	CASSLGLQETQY	2.5	1
18	CANSPTSGDTQY	2.3	1
19	CASSRGPTWQPQH	1.5	1
20-1	CSAMSRGNTIY	1.3	1
20-1	CSAKGLENLPEQY	2.7	1
27	CASSSLDRDNQPQH	1.5	1
27	CASSSTSGVTDTQY	2.3	1

B. HIV-specific CD4+ T cells

TCRBV	CDR3	TCRBJ	%freq
9	CASSVGVRWNSPLH	1.6	10
4-2	CASSQDRGRGTDTQY	2.3	7
15	CATSREQRLAKNIQY	2.4	7
19	CASSSSPTGNIQY	2.4	7
5-1	CASSPSGQIYEQY	2.7	3
7-2	CASSYTGQGLSEAF	1.1	3
7-2	CASSLGPDRAGYT	1.2	3
7-2	CASSLAPRGSPLHF	1.6	3
7-2	CASSLVPNLAKNIQY	2.4	3
19	CASSPVSNQPQH	1.5	3
20-1	CSAPYRAGPHYNEQF	2.1	3
20-1	CSARDVASGDNEQF	2.1	3
27	CASSLRGPANHLMDYGYT	1.2	3
2	CASSLRDAGNTIY	1.3	2
5-1	CASSLGGQGIDGYT	1.2	2
5-1	CASSLTPLWTNEKLF	1.4	2
5-1	CASSLAGYSNQPQH	1.5	2
6-2	CASSQANTQY	2.3	2
7-2	CASSPNGYSNQPQH	1.5	2
7-2	CASSLDFVGNQPQH	1.5	2
7-2	CASSHISRVSSYEQY	2.7	2
7-9	CASSLADSNQPQH	1.5	2
7-9	CASSLDPGPKNIQY	2.4	2
10-2	CASSGRAVIETQY	2.5	2
11-2	CASSLRGNPSSYNSPLH	1.6	2
19	CASSTRDRAEGYT	1.2	2
19	CASSSSPTGIIQY	2.4	2
20-1	CSAQNGPGYT	1.2	2
20-1	CSAILADSYNEQF	2.1	2
20-1	CSAKSYGGGNYNEQF	2.1	2
20-1	CSARDLGARSYNEQF	2.1	2
20-1	CSARGAQGAGEQF	2.1	2
20-1	CSARDPVTNEQF	2.1	2
20-1	CSARRGPRAKNIQY	2.4	2
20-1	CSAKKSTSGSYEQY	2.7	2
27	CASSSPGTGVNIQY	2.4	2
28	CASSSIPTGPLEGTIY	1.3	2

FIG. 6. Clonotypic analysis of HIV- and CMV-specific CD4+ T cells from subject 21. PBMC from subject 21 were stimulated with overlapping Gag peptides or CMV whole antigen in the presence of a CD45/IFN-γ bispecific antibody. Following stimulation for 5 h, cells were extracellularly stained with CD4, CD3, and IFN-γ. IFN-γ-producing cells were sorted into RNAlater followed by unbiased anchored template switch PCR to identify all expressed TCRB genes. freq, frequency.

replicative capacity (10, 30); in addition, transient boosting of CD4+ T-cell responses to HIV Gag peptides has been observed after discontinuation of antiretroviral therapy (28). Overall, these observations suggest that an intimate balance prevails in vivo in which antigenic stimulation drives CD4+ T-cell proliferation but is counteracted by destructive effects mediated by HIV. The mechanistic basis for this destructive effect has been attributed to the phenomenon of preferential infection (11, 14, 15, 17). Indeed, it is clear that HIV infects HIV-specific CD4+ T cells at a higher frequency than CD4+ T cells of other specificities under most circumstances (14). Thus, the presence of HIV-specific CD4+ T cells in infected individuals could be either beneficial, through the provision of cognate help to humoral and CD8+ T-cell responses, or disadvantageous, through the provision of target cells that could serve to amplify HIV replication in vivo. The literature reflects this level of uncertainty, with previous studies providing evidence that could be interpreted as being consistent with either effect (4, 25, 27, 32, 34, 39). In either eventuality, however, a more detailed understanding of both the mechanisms that underlie the process of preferential infection and the consequences of this phenomenon could reveal important insights into HIV pathogenesis.

In the present study, we examined CD4+ T-cell responses to overlapping peptides spanning HIV-1 Gag, Pol, Env, and Nef in a cohort of 23 individuals with chronic HIV infection. Consistent with previous studies, HIV-specific CD4+ T cells in this cohort were generally present at low frequencies (<1% of the total CD4+ T-cell pool) in peripheral blood (Fig. 1). Notably, phenotypic analysis revealed that these HIV-specific CD4+ T cells were relatively immature (Fig. 2 and 3); again, this is consistent with previous observations (17, 45). These data suggest that HIV-specific CD4+ T cells have a shortened life span in vivo compared to CD4+ T cells with other antigen specificities. However, it is important to appreciate that antigen-induced activation per se could lead to CD4+ T-cell death; thus, the low frequency of phenotypically immature CD4+ T cells specific for HIV could simply reflect the consequences of immune activation rather than any direct effects of the virus itself (16). Observations from the two atypical responders in our cohort helped to distinguish between these possible explanations.

The HIV-specific CD4+ T-cell responses in donors 21 and 1 were substantial (>1% of the total CD4+ T-cell pool) and exhibited a mature phenotype exemplified by the expression of CD57, which defines terminal differentiation and replicative senescence in peripheral-blood T cells (5) (Fig. 4). Further, in contrast to findings with the vast majority of infected individuals, the HIV-specific CD4+ T cells of these two donors were not preferentially infected by HIV (Table 2). These observations indicate that HIV-specific CD4+ T cells can accrue to substantial levels in vivo and differentiate fully in the absence of preferential infection. In addition, differences in plasma virus load overall did not appear to account for the profoundly

unusual nature of the HIV-specific $CD4^+$ T-cell responses in these two donors compared with responses of the other individuals in the cohort (Table 1). Thus, preferential infection shortens the life span of HIV-specific $CD4^+$ T cells irrespective of other effects related to viral replication.

To elucidate the mechanistic basis by which preferential infection was circumvented in subjects 21 and 1, we undertook a fine mapping study of individual epitope specificities within the dominant HIV-specific responses and compared the clonotypic architecture of the responding $CD4^+$ T cells to the contemporaneous CMV-specific $CD4^+$ T-cell responses. Remarkably, within a relatively oligoclonal HIV-specific $CD4^+$ T-cell population that was focused almost exclusively on a single Gag-derived epitope, the dominant clonotype also appeared as the dominant CMV-specific $CD4^+$ T-cell clonotype in subject 1 (Fig. 5). This applied to both $CD57^+$ and $CD57^-$ $CD4^+$ T-cell subsets for each specificity; since individual T-cell clonotypes, by definition, are progeny of a single precursor, these data are consistent with a linear maturation process. Similar findings pertained to subject 21, although in this case the responding HIV-specific $CD4^+$ T-cell population was more polyclonal and targeted multiple Gag-derived epitopes (Fig. 6). As most individuals are exposed to CMV within the first 16 years of life, it seems likely that this prevalent clonotype was initially selected and driven to terminal differentiation in the periphery in response to CMV infection even before HIV infection had occurred; the observed cross-reactivity with HIV-1 Gag is, presumably, coincidental. These data suggest, therefore, that preferential infection of HIV-specific $CD4^+$ T cells can be circumvented in the presence of cross-reactive $CD4^+$ T-cell clonotypes driven to maturity by coinfecting viral antigens; this is consistent with previous studies in which it has been shown that terminally differentiated $CD4^+$ T cells are less likely to become infected by HIV in vivo (5). In a broader context, these data demonstrate a biological relevance for T-cell cross-reactivity in humans, a phenomenon that has been reported previously only with murine models (21, 37).

In summary, we have provided strong evidence that the process of preferential infection reduces the life span of HIV-specific $CD4^+$ T cells in vivo. Furthermore, the data indicate that the clonotypic nature of the responding T cells, influenced by the infection history of the individual, can affect this process. While it is clear that a high frequency of terminally differentiated HIV-specific $CD4^+$ T cells is not sufficient in itself to control viral replication, these findings illuminate a basic pathophysiological process that likely impacts the outcome of HIV infection.

ACKNOWLEDGMENT

D.A.P. is a Medical Research Council (United Kingdom) Clinician Scientist.

REFERENCES

1. **Betts, M. R., D. R. Ambrozak, D. C. Douek, S. Bonhoeffer, J. M. Brenchley, J. P. Casazza, R. A. Koup, and L. J. Picker.** 2001. Analysis of total human immunodeficiency virus (HIV)-specific $CD4^+$ and $CD8^+$ T-cell responses: relationship to viral load in untreated HIV infection. J. Virol. **75:**11983–11991.
2. **Betts, M. R., J. P. Casazza, B. A. Patterson, S. Waldrop, W. Trigona, T.-M. Fu, F. Kern, L. J. Picker, and R. A. Koup.** 2000. Putative immunodominant human immunodeficiency virus-specific $CD8^+$ T-cell responses cannot be predicted by major histocompatibility complex class I haplotype. J. Virol. **74:**9144–9151.
3. **Birch, M. R., J. C. Learmont, W. B. Dyer, N. J. Deacon, J. J. Zaunders, N. Saksena, A. L. Cunningham, J. Mills, and J. S. Sullivan.** 2001. An examination of signs of disease progression in survivors of the Sydney Blood Bank Cohort (SBBC). J. Clin. Virol. **22:**263–270.
4. **Boaz, M. J., A. Waters, S. Murad, P. J. Easterbrook, and A. Vyakarnam.** 2002. Presence of HIV-1 Gag-specific IFN-gamma+IL-2+ and CD28+IL-2+ CD4 T cell responses is associated with nonprogression in HIV-1 infection. J. Immunol. **169:**6376–6385.
5. **Brenchley, J. M., B. J. Hill, D. R. Ambrozak, D. A. Price, F. J. Guenaga, J. P. Casazza, J. Kuruppu, J. Yazdani, S. A. Migueles, M. Connors, M. Roederer, D. C. Douek, and R. A. Koup.** 2004. T-cell subsets that harbor human immunodeficiency virus (HIV) in vivo: implications for HIV pathogenesis. J. Virol. **78:**1160–1168.
6. **Brenchley, J. M., N. J. Karandikar, M. R. Betts, D. R. Ambrozak, B. J. Hill, L. E. Crotty, J. P. Casazza, J. Kuruppu, S. A. Migueles, M. Connors, M. Roederer, D. C. Douek, and R. A. Koup.** 2003. Expression of CD57 defines replicative senescence and antigen-induced apoptotic death of CD8+ T cells. Blood **101:**2711–2720.
7. **Casazza, J. P., M. R. Betts, L. J. Picker, and R. A. Koup.** 2001. Decay kinetics of human immunodeficiency virus-specific $CD8^+$ T cells in peripheral blood after initiation of highly active antiretroviral therapy. J. Virol. **75:**6508–6516.
8. **Clerici, M., N. I. Stocks, R. A. Zajac, R. N. Boswell, D. C. Bernstein, D. L. Mann, G. M. Shearer, and J. A. Berzofsky.** 1989. Interleukin-2 production used to detect antigenic peptide recognition by T-helper lymphocytes from asymptomatic HIV seropositive individuals. Nature **339:**383–385.
9. **Day, C. L., N. P. Seth, M. Lucas, H. Appel, L. Gauthier, G. M. Lauer, G. K. Robbins, Z. M. Szczepiorkowski, D. R. Casson, R. T. Chung, S. Bell, G. Harcourt, B. D. Walker, P. Klenerman, and K. W. Wucherpfennig.** 2003. Ex vivo analysis of human memory CD4 T cells specific for hepatitis C virus using MHC class II tetramers. J. Clin. Investig. **112:**831–842.
10. **Deeks, S. G., J. N. Martin, E. Sinclair, J. Harris, T. B. Neilands, H. T. Maecker, E. Hagos, T. Wrin, C. J. Petropoulos, B. Bredt, and J. M. McCune.** 2004. Strong cell-mediated immune responses are associated with the maintenance of low-level viremia in antiretroviral-treated individuals with drug-resistant human immunodeficiency virus type 1. J. Infect. Dis. **189:**312–321.
11. **Demoustier, A., B. Gubler, O. Lambotte, M. G. De Goer, C. Wallon, C. Goujard, J. F. Delfraissy, and Y. Taoufik.** 2002. In patients on prolonged HAART, a significant pool of HIV infected CD4 T cells are HIV-specific. AIDS **16:**1749–1754.
12. **de Wolf, F., I. Spijkerman, P. T. Schellekens, M. Langendam, C. Kuiken, M. Bakker, M. Roos, R. Coutinho, F. Miedema, and J. Goudsmit.** 1997. AIDS prognosis based on HIV-1 RNA, CD4+ T-cell count and function: markers with reciprocal predictive value over time after seroconversion. AIDS **11:**1799–1806.
13. **Douek, D. C., M. R. Betts, J. M. Brenchley, B. J. Hill, D. R. Ambrozak, K. L. Ngai, N. J. Karandikar, J. P. Casazza, and R. A. Koup.** 2002. A novel approach to the analysis of specificity, clonality, and frequency of HIV-specific T cell responses reveals a potential mechanism for control of viral escape. J. Immunol. **168:**3099–3104.
14. **Douek, D. C., J. M. Brenchley, M. R. Betts, D. R. Ambrozak, B. J. Hill, Y. Okamoto, J. P. Casazza, J. Kuruppu, K. Kunstman, S. Wolinsky, Z. Grossman, M. Dybul, A. Oxenius, D. A. Price, M. Connors, and R. A. Koup.** 2002. HIV preferentially infects HIV-specific CD4+ T-cells. Nature **417:**95–98.
15. **Douek, D. C., L. J. Picker, and R. A. Koup.** 2003. T cell dynamics in HIV-1 infection. Annu. Rev. Immunol. **21:**265–304.
16. **Grossman, Z., M. Meier-Schellersheim, A. E. Sousa, R. M. Victorino, and W. E. Paul.** 2002. CD4+ T-cell depletion in HIV infection: are we closer to understanding the cause? Nat. Med. **8:**319–323.
17. **Harari, A., G. P. Rizzardi, K. Ellefsen, D. Ciuffreda, P. Champagne, P. A. Bart, D. Kaufmann, A. Telenti, R. Sahli, G. Tambussi, L. Kaiser, A. Lazzarin, L. Perrin, and G. Pantaleo.** 2002. Analysis of HIV-1- and CMV-specific memory CD4 T-cell responses during primary and chronic infection. Blood **100:**1381–1387.
18. **Iyasere, C., J. C. Tilton, A. J. Johnson, S. Younes, B. Yassine-Diab, R. P. Sekaly, W. W. Kwok, S. A. Migueles, A. C. Laborico, W. L. Shupert, C. W. Hallahan, R. T. Davey, Jr., M. Dybul, S. Vogel, J. Metcalf, and M. Connors.** 2003. Diminished proliferation of human immunodeficiency virus-specific $CD4^+$ T cells is associated with diminished interleukin-2 (IL-2) production and is recovered by exogenous IL-2. J. Virol. **77:**10900–10909.
19. **Kalams, S. A., and B. D. Walker.** 1998. The critical need for CD4 help in maintaining effective cytotoxic T lymphocyte responses. J. Exp. Med. **188:**2199–2204.
20. **Kaufmann, G. R., N. Khanna, R. Weber, L. Perrin, H. Furrer, M. Cavassini, B. Ledergerber, P. Vernazza, E. Bernasconi, M. Rickenbach, B. Hirschel, and M. Battegay.** 2004. Long-term virological response to multiple sequential regimens of highly active antiretroviral therapy for HIV infection. Antivir. Ther. **9:**263–274.
21. **Kim, S. K., M. Cornberg, X. Z. Wang, H. D. Chen, L. K. Selin, and R. M. Welsh.** 2005. Private specificities of CD8 T cell responses control patterns of heterologous immunity. J. Exp. Med. **201:**523–533.
22. **Legac, E., B. Autran, H. Merle-Beral, C. Katlama, and P. Debre.** 1992.

CD4+CD7−CD57+ T cells: a new T-lymphocyte subset expanded during human immunodeficiency virus infection. Blood **79:**1746–1753.

23. **McNeil, A. C., W. L. Shupert, C. A. Iyasere, C. W. Hallahan, J. A. Mican, R. T. Davey, Jr., and M. Connors.** 2001. High-level HIV-1 viremia suppresses viral antigen-specific CD4(+) T cell proliferation. Proc. Natl. Acad. Sci. USA **98:**13878–13883.
24. **Meyaard, L., S. A. Otto, B. Hooibrink, and F. Miedema.** 1994. Quantitative analysis of CD4+ T cell function in the course of human immunodeficiency virus infection. Gradual decline of both naive and memory alloreactive T cells. J. Clin. Investig. **94:**1947–1952.
25. **Ostrowski, M. A., J. X. Gu, C. Kovacs, J. Freedman, M. A. Luscher, and K. S. MacDonald.** 2001. Quantitative and qualitative assessment of human immunodeficiency virus type 1 (HIV-1)-specific CD4+ T cell immunity to gag in HIV-1-infected individuals with differential disease progression: reciprocal interferon-gamma and interleukin-10 responses. J. Infect. Dis. **184:**1268–1278.
26. **Oxenius, A., D. A. Price, P. J. Easterbrook, C. A. O'Callaghan, A. D. Kelleher, J. A. Whelan, G. Sontag, A. K. Sewell, and R. E. Phillips.** 2000. Early highly active antiretroviral therapy for acute HIV-1 infection preserves immune function of CD8+ and CD4+ T lymphocytes. Proc. Natl. Acad. Sci. USA **97:**3382–3387.
27. **Oxenius, A., D. A. Price, M. Hersberger, E. Schlaepfer, R. Weber, M. Weber, T. M. Kundig, J. Boni, H. Joller, R. E. Phillips, M. Flepp, M. Opravil, and R. F. Speck.** 2004. HIV-specific cellular immune response is inversely correlated with disease progression as defined by decline of CD4+ T cells in relation to HIV RNA load. J. Infect. Dis. **189:**1199–1208.
28. **Pitcher, C. J., C. Quittner, D. M. Peterson, M. Connors, R. A. Koup, V. C. Maino, and L. J. Picker.** 1999. HIV-1-specific CD4+ T cells are detectable in most individuals with active HIV-1 infection, but decline with prolonged viral suppression. Nat. Med. **5:**518–525.
29. **Plana, M., F. Garcia, A. Oxenius, G. M. Ortiz, A. Lopez, A. Cruceta, G. Mestre, E. Fumero, C. Fagard, M. A. Sambeat, F. Segura, J. M. Miro, M. Arnedo, L. Lopalcos, T. Pumarola, B. Hirschel, R. E. Phillips, D. F. Nixon, T. Gallart, and J. M. Gatell.** 2004. Relevance of HIV-1-specific CD4+ helper T-cell responses during structured treatment interruptions in patients with CD4+ T-cell nadir above 400/mm3. J. Acquir. Immune Defic. Syndr. **36:**791–799.
30. **Price, D. A., G. Scullard, A. Oxenius, R. Braganza, S. A. Beddows, S. Kazmi, J. R. Clarke, G. E. Johnson, J. N. Weber, and R. E. Phillips.** 2003. Discordant outcomes following failure of antiretroviral therapy are associated with substantial differences in human immunodeficiency virus-specific cellular immunity. J. Virol. **77:**6041–6049.
31. **Price, D. A., S. M. West, M. R. Betts, L. E. Ruff, J. M. Brenchley, D. R. Ambrozak, Y. Edghill-Smith, M. J. Kuroda, D. Bogdan, K. Kunstman, N. L. Letvin, G. Franchini, S. M. Wolinsky, R. A. Koup, and D. C. Douek.** 2004. T cell receptor recognition motifs govern immune escape patterns in acute SIV infection. Immunity **21:**793–803.
32. **Rosenberg, E., J. Billingsley, A. Caliendo, S. Boswell, P. Sax, S. Kalams, and B. Walker.** 1997. Vigorous HIV-1-specific CD4+ T cell responses associated with control of viremia. Science **278:**1447–1450.
33. **Rosenberg, E. S., M. Altfeld, S. H. Poon, M. N. Phillips, B. M. Wilkes, R. L. Eldridge, G. K. Robbins, R. T. D'Aquila, P. J. Goulder, and B. D. Walker.** 2000. Immune control of HIV-1 after early treatment of acute infection. Nature **407:**523–526.
34. **Schrier, R. D., C. A. Wiley, C. Spina, J. A. McCutchan, I. Grant, et al.** 1996. Pathogenic and protective correlates of T cell proliferation in AIDS. J. Clin. Investig. **98:**731–740.
35. **Scriba, T. J., H. T. Zhang, H. L. Brown, A. Oxenius, N. Tamm, S. Fidler, J. Fox, J. N. Weber, P. Klenerman, C. L. Day, M. Lucas, and R. E. Phillips.** 2005. HIV-1-specific CD4+ T lymphocyte turnover and activation increase upon viral rebound. J. Clin. Investig. **115:**443–450.
36. **Seder, R. A., and R. Ahmed.** 2003. Similarities and differences in CD4+ and CD8+ effector and memory T cell generation. Nat. Immunol. **4:**835–842.
37. **Selin, L. K., M. Cornberg, M. A. Brehm, S. K. Kim, C. Calcagno, D. Ghersi, R. Puzone, F. Celada, and R. M. Welsh.** 2004. CD8 memory T cells: cross-reactivity and heterologous immunity. Semin. Immunol. **16:**335–347.
38. **Seth, N., D. Kaufmann, T. Lahey, E. S. Rosenberg, and K. W. Wucherpfennig.** 2005. Expansion and contraction of HIV-specific CD4 T cells with short bursts of viremia, but physical loss of the majority of these cells with sustained viral replication. J. Immunol. **175:**6948–6958.
39. **Staprans, S. I., and M. B. Feinberg.** 2004. The roles of nonhuman primates in the preclinical evaluation of candidate AIDS vaccines. Expert Rev. Vaccines **3:**S5–S32.
40. **Sylwester, A. W., B. L. Mitchell, J. B. Edgar, C. Taormina, C. Pelte, F. Ruchti, P. R. Sleath, K. H. Grabstein, N. A. Hosken, F. Kern, J. A. Nelson, and L. J. Picker.** 2005. Broadly targeted human cytomegalovirus-specific CD4+ and CD8+ T cells dominate the memory compartments of exposed subjects. J. Exp. Med. **202:**673–685.
41. **Wahren, B., L. Morfeldt-Mansson, G. Biberfeld, L. Moberg, A. Sonnerborg, P. Ljungman, A. Werner, R. Kurth, R. Gallo, and D. Bolognesi.** 1987. Characteristics of the specific cell-mediated immune response in human immunodeficiency virus infection. J. Virol. **61:**2017–2023.
42. **Wilson, D. B., D. H. Wilson, K. Schroder, C. Pinilla, S. Blondelle, R. A. Houghten, and K. C. Garcia.** 2004. Specificity and degeneracy of T cells. Mol. Immunol. **40:**1047–1055.
43. **Wu, C. Y., J. R. Kirman, M. J. Rotte, D. F. Davey, S. P. Perfetto, E. G. Rhee, B. L. Freidag, B. J. Hill, D. C. Douek, and R. A. Seder.** 2002. Distinct lineages of T(H)1 cells have differential capacities for memory cell generation in vivo. Nat. Immunol. **3:**852–858.
44. **Younes, S. A., B. Yassine-Diab, A. R. Dumont, M. R. Boulassel, Z. Grossman, J. P. Routy, and R. P. Sekaly.** 2003. HIV-1 viremia prevents the establishment of interleukin 2-producing HIV-specific memory CD4+ T cells endowed with proliferative capacity. J. Exp. Med. **198:**1909–1922.
45. **Yue, F. Y., C. M. Kovacs, R. C. Dimayuga, P. Parks, and M. A. Ostrowski.** 2004. HIV-1-specific memory CD4+ T cells are phenotypically less mature than cytomegalovirus-specific memory CD4+ T cells. J. Immunol. **172:**2476–2486.