HIV-1 Reverse Transcriptase-Specific CTL Against Conserved Epitopes Do Not Protect Against Progression to AIDS[1]

Sjoerd H. van der Burg,[2*] Michèl R. Klein,[2†] Oscar Pontesilli,[‡] Agnes M. Holwerda,[†] Jan-Wouter Drijfhout,[*] W. Martin Kast,[*§] Frank Miedema,[†‡] and Cornelis J. M. Melief[3*]

A small group of HIV-1-infected subjects who either do not progress to AIDS or progress only slowly have sustained HIV-1-specific CTL responses. It has been suggested that the specificities of these responses differ from the CTL responses of rapid progressors due to recognition of epitopes that are under structural or functional constraints. We have, in this respect, studied the CTL response to reverse transcriptase (RT) in long term survivors (LTS) and in HIV-1-infected individuals who progressed to AIDS within 3 to 6 yr. Both LTS and progressors displayed vigorous RT-specific CTL responses of comparable magnitude during the asymptomatic phase. From each individual at least two CTL lines were obtained from blood samples drawn at different time points during follow-up. A total of 19 CTL lines recognized nine different RT-derived epitopes. CTL obtained from progressors recognized epitopes with a similar degree of amino acid conservation as epitopes targeted by CTL from LTS. Furthermore, five of seven epitopes were recognized by both LTS and progressors. Moreover, one of the epitopes recognized by progressors contained the highly conserved YMDD motif that is essential for RT activity. In conclusion, our data imply that neither the magnitude nor the specificity of HIV-1-specific CTL against RT is a major cause of a more protracted course of disease. *The Journal of Immunology,* **1997, 159: 3648–3654.**

Virus-specific CTL are believed to control and are sometimes able to clear most viral infections. The decrease in HIV-1 viral load following the appearance of HIV-1-specific CTL during primary HIV-1 infection (1), a temporal association between persistent CTL activity and stable viral load in the asymptomatic phase (2), together with the demonstration that CTL efficiently suppress HIV-1 replication in vitro (3) are thought to reflect the efficacy of HIV-1-specific CTL.

A minority of HIV-1-infected subjects do not progress to AIDS, although they have been infected with HIV-1 for >10 yr. These so-called long term survivors (LTS)[4] maintain persistent and broadly directed CTL responses with low numbers of HIV-1-infected cells. In contrast, most infected persons lose HIV-1-specific CTL activity during progression to AIDS, and this is paralleled by an increase in viral load (2, 4–9). This suggests that HIV-1-specific CTL may be involved in delaying progression to AIDS. The HIV-1-specific T cell response of LTS may be distinct from that of progressors in several aspects. First, LTS might display higher levels of peripheral expansion of HIV-1-specific CTL, resulting in the generation of more effector cells. Another possibility is that CTL from LTS target more viral proteins (i.e., epitopes) at the same time. As for the latter, it has been shown that subjects who progressed to AIDS displayed strong HIV-1-specific CTL responses against all major proteins during their asymptomatic phase[5] (2, 10). It seems therefore unlikely that the magnitude or the breadth of the CTL response discriminates long term survival from progression to AIDS. Another possibility could be the biophysical properties of the CTL-targeted epitopes that impact on the outcome of disease. LTS might recognize epitopes that are expressed early in the life cycle of the virus and kill infected cells before new virions are released. Alternatively, epitopes that do not display sequence variation because of functional or structural constraints might be recognized, which makes it difficult for the virus to escape from CTL (7, 11–17).

Hitherto many CTL epitopes have been described but their clinical importance has not been established, since clinical follow-up data for most patients have not been reported. In addition, sequences of some proteins (e.g., Env) in laboratory HIV-1 strains might have little homology with those that affected these HIV-1-infected individuals. We wanted to study whether qualitative differences exist in epitopes recognized by CTL from LTS and progressors. For this purpose we studied the CTL response to reverse transcriptase (RT), since this protein exhibits minor variation among sequenced isolates and is functionally essential for viral replication. Moreover, RT is a major target for virus-specific CTL at various stages of HIV-1 infection (18). For these reasons, sequence variation in RT-derived CTL epitopes would interfere with HIV-1 replication. We made an inventory of viral epitopes recognized by immunodominant CTL directed against RT in a group of HIV-1-infected individuals consisting of four LTS (asymptomatic

*Department of Immunohematology and Blood Bank, University Hospital, Leiden; †Department of Clinical Viro-Immunology, Central Laboratory of The Netherlands Red Cross Blood Transfusion Service; and ‡Department of Human Retrovirology, Academic Medical Center, Amsterdam, The Netherlands; and §Cardinal Bernadin Cancer Center, Loyola University of Chicago, Maywood, IL 60153

Received for publication March 7, 1997. Accepted for publication July 9, 1997.

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[1] This work was supported by the Dutch Aids Fund (Stichting AIDS Fonds) on advice of the Dutch Program Committee of AIDS Research (95-017) in the context of the National AIDS Research Stimulation Program.

[2] Both authors contributed equally to the work described.

[3] Address correspondence and reprint requests to Dr. C. J. M. Melief, Department of Immunohematology and Blood Bank, University Hospital, P.O. Box 9600, 2300 RC Leiden, The Netherlands. E-mail address: BURG_S@rullf2.Medfac.LeidenUniv.nl

[4] Abbreviations used in this paper: LTS, long term survivor; RT, reverse transcriptase; rVV, recombinant vaccinia virus; B-LCL, B lymphoblastoid cell line; CTLp, cytotoxic T lymphocyte precursor; PCP, pneumocystic carinii pneumonia.

[5] O. Pontesilli, M. R. Klein, S. Kerkhof-Garde, N. Pakker, F. de Wolf, H. Schuitemaker, and F. Miedema. 1997. Kinetics of HIV-1 specific cytotoxic T lymphocyte responses and viral load in the natural history of HIV-1 infection. *Submitted for publication.*

0022-1767/97/$02.00

>10 yr, with normal $CD4^+$ T cell counts) and three (rapid) progressors from the Amsterdam Cohort Studies on HIV-1 infection and AIDS. No differences were found between these individuals in either the magnitude of the total RT-specific CTL response or in the level of conservation of the epitopes.

Materials and Methods

Study population

The Amsterdam Cohort Studies on HIV Infection and AIDS were initiated in October 1984 (19). Data for cohort patients were collected every 3 mo in visits that consisted of a standardized medical history and collection of blood samples for HIV-1 serology and cellular immunology. The date of entry for participants already HIV-1 seropositive at enrollment or the date of documented HIV-1 seroconversion, taken as the midpoint between the last seronegative and the first HIV-1 seropositive visit, was used as the reference point for clinical follow-up. For this study, seven cohort participants were studied: three patients who progressed to AIDS within 3 to 6 yr after seroconversion (P039, P356, and P1211) and four participants who had 10 to >11 years of asymptomatic follow-up and $CD4^+$ T cell counts >500 cells/mm^3 (L016, L090, L658, and L709).

Viral markers, comprising serum HIV-1 RNA levels, and viral load, measured as the frequency of circulating $CD4^+$ T cells productively infected with HIV-1, were determined as previously described (2).

Recombinant vaccinia viruses (rVV)

The rVV used in these studies were constructed from the Copenhagen strain of vaccinia virus and include rVV TG.4163 expressing RT of HIV-1_{LAI} and control-rVV 186-poly containing no insert (provided by Dr. Y. Rivière (Pasteur Institute, Paris, France) and Dr. M. P. Kieny (Transgène S.A., Strasbourg, France)).

Expansion of HIV-1 RT-specific CTL responses in vitro

Primed HIV-1-specific CTL precursors were expanded in vitro using a previously described method (2, 16) and that does not result in the expansion of naive Ag-specific CTL (M. Klein, unpublished observations). Briefly, PBMC isolated and cryopreserved at different time points during the study were thawed and resuspended in RPMI 1640 supplemented with antibiotics and 10% pooled human serum. Eight serial dilutions of PBMC ranging from 745 to 20,000 cells/well were seeded in 24 replicate wells in 96-well round-bottom microtiter plates. Stimulator cells were autologous EBV-transformed B lymphoblastoid cell lines (B-LCL) infected with 5 multiplicity of infection rVV-TG.4163 and subsequently inactivated with 1% paraformaldehyde. To each well, 10^4 fixed stimulator cells and 10^4 autologous PBMC (30 Gy irradiated) were added, and microcultures were maintained for 15 days at 37°C in 5% CO_2. On days 2 and 9, cultures were fed medium containing 10 U/ml rIL-2 (kindly provided by Dr. R. Rombouts, Chiron B.V., Amsterdam, The Netherlands), and on day 7, they were restimulated with 10^4 fixed stimulator cells and rIL-2 (10 U/ml). On day 15, wells were split, and effector cells were tested for cytotoxicity. All RT-specific lymphocyte cultures from the various time points were expanded by nonspecific restimulation with 2×10^4 irradiated (30 Gy) PBMC/well and 5000 irradiated (50 Gy) B-LCL/well and medium containing rIL-2 (10 U/ml) and 1 μg/ml leukoagglutinin (PHA-leukocytes, Sigma Chemical Co., St. Louis, MO) and tested for cytotoxicity. Cultures from wells displaying a continuous high specific cytotoxicity for the endogenously processed Ag were expanded and tested for epitope specificity using peptide-pulsed autologous B-LCL as target cells.

Peptides

A set of 536 peptides spanning the entire sequence of HIV-1_{LAI} RT (20) was synthesized as 10-amino acid-long peptides with an overlap of nine amino acids (e.g.: P1, PISPIETVPV; P2, ISPIETVPVK; P536, VPAHKG IGGN). Peptides were synthesized by solid phase strategies on an automated multiple peptide synthesizer (Abimed AMS 422, Langenfeld, Germany) using F-moc chemistry. Peptides were analyzed by reverse phase HPLC, dissolved in DMSO, and aliquoted. For cytotoxicity assays, peptides were diluted in 0.9% NaCl to a peptide concentration of 100 μg/ml and stored in 96-well V-bottom plates at −70°C.

Cytotoxicity assays

Standard ^{51}Cr release assays were performed as previously described (16). Briefly, autologous B-LCL were infected with 5 multiplicity of infection rVV-TG.4163 or rVV 186-poly and labeled with 100 μCi of $Na_2{}^{51}CrO_4$ (Amersham International, Aylesbury, U.K.) for 16 h. After three washes, 4×10^3 target cells were added to each well. After 4 h, supernatants were harvested, and radioactivity was counted with a gamma counter (Cobra II, Packard Instruments Co., Meriden, CT). Specific lysis (percentage) was calculated with the formula: 100 × ([experimental release − spontaneous release] ÷ [maximum release − spontaneous release]). Spontaneous ^{51}Cr release was always <15% of maximum release.

Fine mapping of epitopes was performed by thawing the 96-well V-bottom plates containing the 10-mer peptides and transfer of 5 μl from each well to 96-well U-bottom plates before addition of target cells. Autologous ^{51}Cr-labeled target cells (4×10^3) were added in 45 μl and preincubated for 30 min at room temperature before the effector cells were added. The final concentration of peptide was 10 μg/ml.

CTLp frequencies were determined as described previously (2). Briefly, wells were considered positive when ^{51}Cr release exceeded 10% specific lysis. Statistical analysis was performed using methods previously described by Strijbosch et al. (21). CTLp frequencies are expressed as the number of CTLp per 10^6 PBMC. RT-CTLp frequencies were computed as differences between CTLp frequencies determined on RT-expressing vs control targets.

Results

Analysis of RT-specific CTL response during the natural history of HIV-1 infection

We studied four LTS and three cohort participants who progressed to AIDS within 3 to 6 yr after HIV-1 seroconversion. The three progressors in our study showed a precipitous decline in $CD4^+$ T cell counts during progression (data not shown). Clinical data for the studied subjects are presented in Table I.

All seven HIV-1-positive individuals showed significant RT-specific CTL-mediated immune responses during their asymptomatic period (Fig. 1). Subject P039 showed a strong CTL response against RT in the first 18 mo after HIV-1 seroconversion, with CTLp frequencies ranging from $300/10^6$ to a peak of $4500/10^6$ PBMC at 18 mo. Thereafter, CTLp frequencies declined rapidly to undetectable levels. Subject P356 showed a strong CTL response, with CTLp frequencies in the range of $1000/10^6$ PBMC for 6 yr, which then declined to undetectable levels. Of note, P356 developed pneumocystic carinii pneumonia (PCP) 3 yr after seroconversion. $CD4^+$ T cell counts then stabilized at ±200 cells/mm^3 (data not shown), and CTL activity persisted up to 3 yr after PCP. Subject P1211 showed an RT-specific CTL response with CTLp frequencies ranging from $20/10^6$ PBMC at seroconversion to a peak at 500 CTLp/10^6 PBMC at 18 mo. This subject also showed a decline in RT-specific CTLp, which became undetectable after diagnosis of AIDS (Fig. 1*A*).

Analysis of the CTLp frequencies of all three LTS revealed that they maintained persistent RT-specific CTL responses during >10 yr of follow-up. Subjects L016 and L709 showed a stable RT-specific CTL response, with CTLp frequencies in the range of 20 to $200/10^6$ PBMC. Subjects L090 and L658 showed CTLp frequencies between 200 and $1000/10^6$ PBMC (Fig. 1*B*).

Identification of epitopes in RT recognized by CTL from LTS

Bulk cultures of CTL were expanded from all RT-specific wells of limiting dilution experiments performed to determine CTLp frequencies and tested for recognition of recombinant vaccinia-RT_{LAI}-infected autologous B cell targets. To avoid skewing of the T cell repertoire toward a particular epitope, positive CTL bulk cultures were not cloned but were nonspecifically expanded for 2 to 3 wk and subsequently retested against rVV-infected B-LCL and peptide-loaded B-LCL. Propagation of bulk cultures to the number of T cells necessary to scan the entire RT protein resulted in many cases in the outgrowth of nonspecific T cells. However, for each HIV-1-infected individual we succeeded in independently procuring RT-specific CTL populations from at least two PBMC samples obtained at different time points.

Table 1. *Clinical data of LTS and progressors*

	HLA Class I	CC-R5 Genotypes	Viral Factors	HIV-1 Seroconversion Date	Age at Seroconversion	(Non-)Syncitium Inducing ((N) SI)	AIDS Diagnosis	Event	Serum HIV-1 RNA Copies/ml[a]	TCID/10^6 $CD4^+$ T Cells[b]	Follow-up (Years)
LTS											
L016	A*0201,29;B44,51 Cw2,5	WT/Δ32		01-23-85	29	NSI			10^3–$10^{4.56}$	2–17	>11.5
L090	A1,*0201;B41,*5701 Cw*0602,Cw*1701	WT/WT		02-01-85	41	NSI			$<10^3$	<10	>11.5
L658	A1,*0201;B*0801,61 Cw2,Cw7	WT/WT		09-25-86	37	NSI			$<10^3$[c]	<10	>10.0
L709	A1,*6802;B15,*5701 Cw*0602,Cw7	WT/WT	Deletions in *nef* and *v pu* genes	10-04-85	29	NSI			10^3–$10^{3.82}$	<10	10.0
Progressors											
P039	A1, *0201;B*0801,44 Cw5,Cw7	WT/WT		10-30-87	36	SI	11-20-90	CAQ	$10^{4.49}$–$10^{5.83}$	<10–323	3.1
P356	A*0201,28;B27,38 Cw2	WT/WT		04-03-85	37	NSI	07-25-88	PCP	$10^{3.85}$–$10^{5.36}$	5–65	3.3
P1211	A*0201,29;B44;Cw4	WT/WT		03-17-87	26	NSI	01-11-93	CM	10^3–$10^{5.92}$[d]	15–177	5.5

[a] Range of serum HIV-1 RNA levels during follow-up.
[b] Range of viral load when measured as the frequency of productively HIV-1-infected circulating $CD4^+$ T cells during follow-up.
[c] Serum RNA levels dropped below 10^3 copies/ml after initial 2.32-log reduction.
[d] An initial 5-log reduction to $<10^3$ copies/ml coincided with high levels of RT-specific CTL.

From the four LTS studied, 12 CTL lines were obtained that could be used to scan the entire RT protein to determine the epitope specificity. This was performed by testing CTL against a set of 536 peptides, 10 amino acids long and overlapping by nine amino acids, spanning the entire RT protein of HIV-1_{LAI}. A total of six different epitopes were identified. Two CTL of L016, obtained from samples taken 6 yr apart, recognized only one peptide, EELRQHLLRW (Fig. 2*A*). All three CTL of L090 recognized a single peptide, PIVLPEKDSW (Fig. 2*B*), whereas the four CTL of L658 recognized two different peptides, SPIETVPVKL and EILKEPVHGV (Fig. 2*C*). From L709, three CTL were obtained that recognized two different peptides, KITTESIVIW and PIVLP EKDSW, the latter of which was shared with L090, and a set of five overlapping peptides that spans the sequence IVGAETFY VDGAAS (Fig. 2*D*). Two of the recognized peptides derived from RT had already been defined by others (Table II; peptide 308, EILKEPVHGV; peptide 434, IVGAETFYVDGAAS).

Identification of epitopes in RT recognized by CTL from progressors

From all three progressors studied we were able to establish seven CTL lines: three from P039, two from P356, and two from P1211. These RT-specific CTL lines recognized a total of five different peptides. The three CTL lines of P039 all recognized a different peptide. Peptide TWETWWTEYW was newly identified; the other two peptides, EELRQHLLRW and EILKEPVHGV, were also recognized by CTL from LTS L016 and L658 (Fig. 3*A*). CTL of P356 recognized peptide IYQYMDDLYV (Fig. 3*B*), and CTL of P1211 recognized peptides RETKLGKAGY and TWETWWTEYW, of which the latter peptide was also recognized by P039 (Fig. 3*C*). Thus, a total of five peptides were recognized, three of which were newly identified. The other two peptides (peptide 180, IYQYMD DLYV; peptide p308, EILKEPVHGV) had been identified as being recognized by LTS in two other studies describing RT-derived epitopes (Table II and III).

Putative HLA class I restriction elements for RT-specific CTL

CTL reacted with the overlapping peptides when the allele-specific C-terminal anchor residue appeared in the sequence. Sometimes,

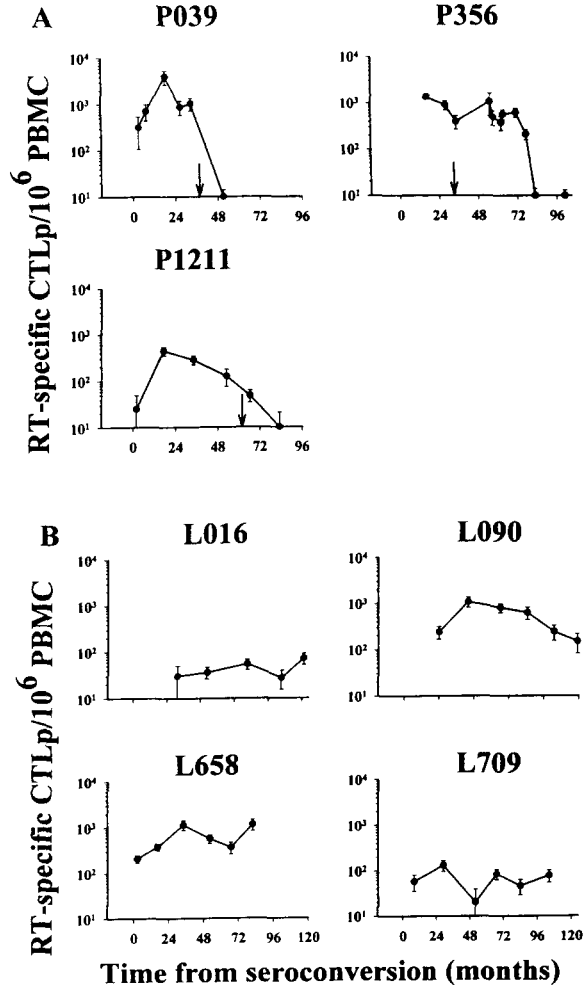

FIGURE 1. *A*, Progressors with high levels of RT-specific CTL activity during the asymptomatic phase. Diagnosis of AIDS is indicated by the arrow. *B*, LTS with persistent levels of HIV-1 RT-specific CTL activity.

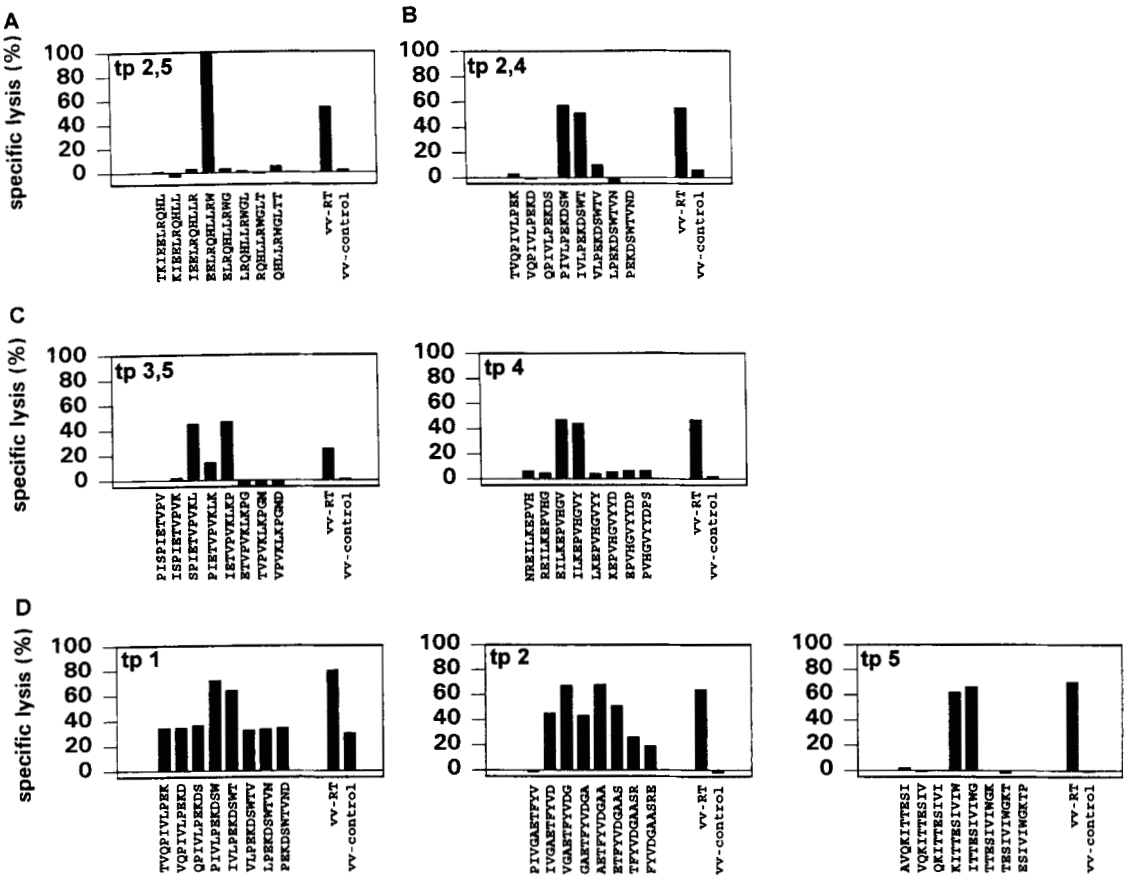

FIGURE 2. RT-derived epitopes recognized by four LTS at different time points (*A*, L016; *B*, L090; *C*, L658; *D*, L709). The time point (tp) indicates from which time point sample CTL were obtained; tp1 corresponds with the first symbol of Figure 1, and tp2,3 indicates that CTL obtained from time points 2 and 3 recognized the same peptide. vv-RT, target cells infected with rVV TG.4163 (RT); vv-control, target cells infected with rVV 186-poly (control). Shown is the median specific lysis of triplicate wells. Effector and target cells were incubated at an E:T ratio of 3:1.

Table II. *Conservation of CTL epitopes recognized in HIV-1 RT*

Peptide No.	Sequence	Recognized by		Conservation and Functional Constraints	MHC Class I Restriction Element[a]	Reference
		LTS	Progressor			
003	SPIETVPVKL[b]	+		SPIetVPVKL[c]	(A2/B61)	This study
180	IYQYMDDLYV	(+)[d]	+	IyQYmDDLYV	A*0201	(22)/this study
203	EELRQHLLRW	+	+	EeLRqHLlrW	B44	This study
243	PIVLPEKDSW	+	(+)[e]	pIvLPeKdsW	B*5701	This study
308	EILKEPVHGV	+	+	EiLKePVHgV	A*0201	(23)/this study
374	KITTESIVIW	+	(+)[e]	kittEsIvIW	B*5701	This study
397	TWETWWTEYW		+	TWetWWteYw	B44	This study
434–438	IVGAETFYVDGAAS	+		IvGAETfYVDGAAs	A*6802	(24)/this study
448	RETKLGKAGY		+	ReTklGKAGY	A29	This study

[a] MHC class I restriction elements were identified by comparison of peptide sequences with known peptide-binding motifs (25) or by overlap in HLA types of individuals recognizing this peptide.

[b] Amino acid sequence according to HIV-1_{LAI} (20). Sometimes two overlapping peptides were recognized; in that case, the peptide most likely containing the complete CTL epitope is given.

[c] Residues that are conserved in all clades are printed in capitals, and other residues are printed in lower case. Residues known to be important for RT function are underlined (20, 26).

[d] This CTL epitope was recognized by a LTS in the study of Harrer et al. (22) and by a progressor in our study.

[e] These two epitopes were recognized by an HLA-B*5701 positive progressor who was studied for the recognition of these two epitopes especially.

but not always, two or three deca-peptides were recognized sequentially, depending mostly on the presence of the N-terminal anchor. The CTL epitope located within peptide 180 (IYQYMD DLYV) has been reported to be recognized by HLA-A*0201-restricted CTL from LTS (22); this HLA type was shared with progressor P356 in our study. CTL from HLA-A2-positive individuals L658 and progressor P039 both recognized peptide 308, which had been shown to be restricted by HLA-A*0201 (23). LTS L016 as well as progressor P039 shared the HLA-B44 molecule, and peptide 203, which was recognized by both, fit the HLA-B44 peptide binding motif (24). Progressors P039 and P1211 recognized peptide 397, which also fit the HLA-B44 motif. Most likely, these peptides were presented by HLA-B44. Peptide 243 (PIVLPE KDSW), which was recognized by CTL from L090 and L709, was

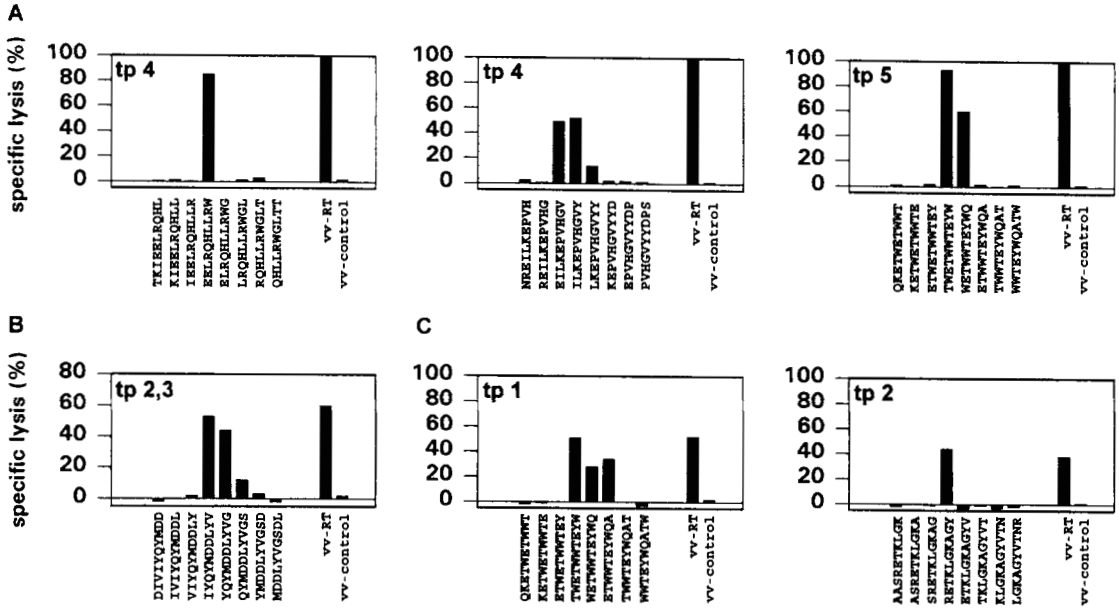

FIGURE 3. RT-derived epitope recognized by the three progressors at different time points (*A*, P039; *B*, P356; *C*, P1211). For explanatory notes, see Figure 2.

Table III. *Degree of conservation of published HIV-1 reverse transcriptase-derived CTL epitopes*

Sequence	Amino Acid Numbers	HLA Restriction and Reference		Recognized by:
GPkVKQWPL[a]	RT 17–25	B8	23	Asymptomatic
VLDVGDaYFSv[b]	RT 107–117	A2.1	17 (W. Herr, personal communication)	Healthy blood donor/asymptomatic
WKGSPAIFQsSMT	RT 153–165	B7	20	?
AIFQsSMTk	RT 158–166	A3.1,11,33	23, 27	Asymptomatic
hPdivIyQY	RT 175–183	B35	20	LTS
vIyQYmDDL	RT 179–187	A2.1	22	LTS
tkiEeLRqHLlr	RT 200–211	Bw60	23	Asymptomatic
LvgkLNWASQIY	RT 260–271	Bw62	28	?
iLKePVHgV	RT 309–317	A2.1	23	Asymptomatic
iLKePVHgVY	RT 309–318	Bw62	28	?
iyQepfKnLKTg	RT 341–352	All	23	Asymptomatic
PiqkETWetW	RT 292–401	A32	7	LTS/Progressor
EkePIvGAETfYVDGAAnReTrlGK	RT 430–454	A28	24	?

[a] Residues that are conserved in all clades are printed in capitals, and other residues are printed in lower case.
[b] Residues known to be important for RT function are underlined.

HLA-B*5701 restricted. The optimal peptide lacked the N-terminal proline (data not shown). One of the other CTL of L709 recognized peptide 374 (KITTESIVIW). This peptide also bound to HLA-B*5701 (our unpublished observations). As previously mentioned, most CTL reacted with one or two 10-amino acid-long peptides containing the CTL epitope. One of the CTL from LTS L709 recognized at least five partially overlapping peptides (Fig. 2*D*). A sequence containing these peptides was reported to be restricted by HLA-A28 (25) (Table III). Since subject L709 also displayed the HLA-A*6802 molecule, which is a subtype of HLA-A28, this molecule is probably the restricting element. Peptide 448 fit the recently published HLA-A29 peptide binding motif; indeed, this HLA molecule was also displayed by subject P1211 (26).

Level of conservation of RT-derived CTL epitopes

Among the published viral isolates, RT is the most conserved protein of HIV-1 (20). This protein is essential for viral replication, and as such, the amino acid sequence conservation is thought to reflect both structural and functional constraints. This means that alterations in particular amino acids will result in loss of function or structure that probably leads to replication incompetent or defective viruses. It can be hypothesized that differences in the properties of the RT-derived CTL epitopes chosen by the immune system may account for a differential disease course, since mutations in less constrained epitopes may readily result in escape and subsequently reduced viral control (17).

Inspection of the amino acid sequence of the nine RT-derived CTL epitopes showed that there was no difference in their degree of conservation (Table II). These epitopes contained one to five residues that were not conserved compared with the sequenced viral isolates known to date. For example, peptide 243, recognized by CTL from LTS L090 and L709, and peptide 397, recognized by CTL from progressors P039 and P1211, both contain five nonconserved residues (Table II). In addition, LTS and progressors similarly recognized a peptide containing only one nonconserved residue (peptide 308; Table II). Furthermore, progressor P356 recognized peptide 180, of which eight of nine residues have been reported to be under functional constraint. P356 developed AIDS

within 3 yr, demonstrating that recognition of this epitope is by itself not protective.

Next to the level of amino acid conservation, LTS might recognize a distinct set of peptides, resulting in prolonged asymptomatic survival. Two out of the nine epitopes were recognized by CTL from LTS as well as from progressors in the seven HIV-1-positive individuals tested here. Additionally, one of the epitopes recognized by progressor P356 was also reported to be recognized by CTL from a LTS reported by Harrer et al. (22). Two of the HLA-B*5701-restricted peptides (peptides 243 and 374) recognized by CTL from LTS L090 and L709 were also recognized by CTL from an HLA-B*5701-positive progressor (Table II) (our unpublished observations). Recently, Goulder et al. reported two HIV-1*gag*-derived HLA-B57-restricted CTL epitopes that were recognized by LTS (27). Both epitopes were recognized by HLA-B57-restricted CTL from a rapid progressor, as reported previously by Johnson et al. (28). Thus, of these 11 epitopes, a total of seven were targets for CTL of both LTS and progressors. This suggests that epitopes recognized by CTL from both groups were not unique to either group.

Discussion

This study was designed to explore unique features of HIV-1-specific CTL of LTS. It was confirmed that neither the magnitude of the CTL response nor the number of target proteins or epitopes recognized distinguished LTS from rapid progressors (see Footnote 5) (2, 7). Therefore, we evaluated the hypothesis that the quality of the CTL epitopes could be relevant for the patients rate of disease progression. Our data showed that all LTS and progressors studied here displayed strong RT-specific CTL responses during their asymptomatic phase. We observed that the CTLp frequencies of two LTS (L016 and L709) were consistently lower. Interestingly, L016 contained a 32-bp deletion in the β-chemokine receptor CC-R5, and in L709, all virus isolates contained a 12-bp deletion in *nef*, which in both cases might have resulted in a lower number of CTL needed to control infection (29, 30).

Using a method that allows for nonspecific expansion of primed T cells, we were able to expand at least two RT-specific CTL lines from different time points during the asymptomatic phase from each individual. A total of 19 different CTL lines identified nine different CTL epitopes. When the number of amino acid residues varied between the different described viral isolates was taken as a measure of conservation of the CTL epitope, no differences could be found in the degree of conservation between CTL epitopes recognized by CTL from LTS and those from progressors. Both groups recognized peptides with one to five nonconserved residues (Table II). Furthermore, addition of previously reported epitopes (Table III) clearly shows that the level of conservation of CTL epitopes does not discriminate between LTS and progressors. Moreover, one epitope that is known to be under functional constraint, IYQYMDDLYV, was recognized by an HIV-1-infected individual who progressed within 3 yr to AIDS. In contrast, others have reported that CTL from an LTS who remained asymptomatic for >18 yr recognized this epitope (22). Clearly, recognition of this epitope did not protect against progression to AIDS. However, true progression might have been delayed, since subject P356 maintained RT-specific CTL for about 3 yr and survived for 6 yr after the diagnosis of PCP. Alternatively, the difference in disease progression between LTS and progressors might be related to a unique set of CTL epitopes recognized by LTS. Five of seven identified epitopes were recognized by both LTS and progressors in this study. Moreover, despite recognition of these peptides, a rapid progression to AIDS was noted in those individuals who progressed. Our results extend those of Harrer et al., who noted that three *gag* and one RT-derived CTL epitopes were recognized not only by LTS, but by progressors as well (7). Taking into account that two previously identified HLA-B57-restricted *gag*-derived epitopes were also recognized by CTL from LTS (27) and rapid progressor 010-063j in the study of Johnson et al. (28), a total of 11 CTL epitopes, six of which were identified in RT, were recognized by both LTS and progressors. This demonstrates that there is no distinct set of CTL epitopes in RT or *gag* recognized by LTS.

The question that remains is what makes an LTS? Since the time-to-AIDS appears to follow a Gaussian distribution, LTS represent the tail end of the normal distribution. This is most likely due to favorable combinations of viral parameters, host genetics, antiviral immune responses, and environmental cofactors. One should anticipate that individual elements of these favorable combinations might be found in each HIV-1-infected person. The magnitude, breadth, quality, and timing of the CTL response are among the features of HIV-1-specific CTL responses held responsible for efficient control of HIV-1 infection. It is clearly demonstrated that neither the magnitude of the CTL response nor the breadth or the presence of a strong HIV-1-specific CTL response directed against epitopes under structural or functional constraints guarantees long term survival. One of the unexplored factors that might influence the course of disease is the timing of the onset of HIV-1-specific CTL responses upon primary infection. Early control of HIV-1 infection, even before HIV-1 is disseminated throughout the whole body, might extend the asymptomatic period or, in some rare cases, lead to sterilizing immunity. Frequently exposed, seronegative individuals have been reported who display HIV-1-specific T cell responses in the absence of clear-cut infection (31, 32). The timing of the immune response might also explain the results found in studies describing the resistance to HIV-1 in individuals already infected with HIV-2 (33, 34). HIV-2 appears to be less transmissible and less pathogenic (35) than HIV-1, and in the Gambian prostitute cohort, CTL were found that were highly cross-reactive with HIV-1 and HIV-2. This suggests that priming with a less virulent (i.e., HIV-2) virus strain might activate CTL able to recognize and abolish HIV-1 at transmission. HIV type 1 and 2 cross-reactive CTL that are at the host's disposal might deal quickly with freshly entered HIV-1. A cross-priming experiment in monkeys showed that indeed protection can be achieved following priming with a less pathogenic virus (36).

Certain HLA molecules are frequently found in LTS (e.g., HLA-B27,B51&B57), whereas others are found more frequently in progressors (e.g., HLA-A24,B8,B35&Cw4) (37). This suggests that CTL responses restricted by certain HLA alleles may have a different impact on disease progression. Two of the RT-derived CTL epitopes recognized by LTS, L090 and L709, were presented in the context of HLA-B57. While these epitopes were also recognized by a progressor, this does not exclude that other HIV-1 CTL epitopes are presented by HLA-B57 that significantly slow down disease progression. Whether such HLA-B57 epitopes exist in LTS is currently under investigation.

In conclusion, although it is generally accepted that CTL contribute to the control of HIV-1 replication, our data add to the growing idea that LTS do not differ from progressors with respect to the magnitude of the HIV-1-specific CTL responses, in the choice of target proteins, or in the functional quality of CTL epitopes recognized. Up until now, LTS were studied as a relatively homogeneous group of individuals who have remained asymptomatic for a long time. It is likely that favorable combinations of different factors (e.g., timing, breadth, magnitude, and specificity of CTL response; intact T helper responses; and host

and viral genetic factors) contribute to long term survival. Therefore, recording of genetic factors and clinical data of the HIV-1-infected subjects should be combined with full characterization of HIV-1-specific CTL responses together with the corresponding in vivo CTL epitope sequence variation data at different clinical stages of infection to determine which properties of the HIV-1-specific CTL response contribute to survival.

Acknowledgments

The authors are indebted to Willemien Benckhuijsen for synthesis of all peptides, to Drs. Stephen Schoenberger and Dawn Clark for carefully reading this manuscript, to the participants in the Amsterdam Cohort Studies on HIV Infection and AIDS, without whom this study would not be possible, to Dr. F. deWolf for providing RNA load data, and to Dr. H. Schuitemaker for CC-R5 typing.

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