Study of Antigen-Processing Steps Reveals Preferences Explaining Differential Biological Outcomes of Two HLA-A2-Restricted Immunodominant Epitopes from Human Immunodeficiency Virus Type 1

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Cytotoxic T-lymphocyte (CTL) responses directed to different human immunodeficiency virus (HIV) epitopes vary in their protective efficacy. In particular, HIV-infected cells are much more sensitive to lysis by anti-Gag/p17(77-85)/HLA-A2 than to that by anti-polymerase/RT(476-484)/HLA-A2 CTL, because of a higher density of p17(77-85) complexes. This report describes multiple processing steps favoring the generation of p17(77-85) complexes: (i) the exact COOH-terminal cleavage of epitopes by cellular proteases occurred faster and more frequently for p17(77-85) than for RT(476-484), and (ii) the binding efficiency of the transporter associated with antigen processing was greater for p17(77-85) precursors than for the RT(476-484) epitope. Surprisingly, these peptides, which differed markedly in their antigenicity, displayed qualitatively and quantitatively similar immunogenicity, suggesting differences in the mechanisms governing these phenomena. Here, we discuss the mechanisms responsible for such differences.

The mechanisms of presentation of intracellular antigens by major histocompatibility class I (MHC-I) molecules are well documented, and several of these steps have been described previously (28). First, antigenic proteins are degraded in peptides by different cytosolic complexes, the most highly implicated and frequently described being the proteasome. Second, peptides are translocated by the transporter associated with antigen-processing (TAP) into the endoplasmic reticulum (ER), where they bind newly synthesized empty MHC-I molecules. Third, stable peptide/MHC-I complexes are exported via the Golgi apparatus to the cell surface, where they can be recognized by CD8⁺ T cells expressing a specific T-cell receptor. However, the steps in antigen processing which determine a dominant T-cell response are still poorly understood. Elucidating the mechanisms that determine the antigenicity of epitopes for MHC-I presentation is critical for defining targets for peptide-designed vaccines or immunotherapies that limit viral spread.

In the present work, we investigated the processing of two HLA-A2-restricted epitopes, p17(77-85), from the Gag/p17 matrix protein, and RT(476-484), from the Pol/reverse transcriptase (RT) complex, which are naturally presented by human immunodeficiency virus (HIV)-infected cells (35). Previous studies have shown that p17(77-85)-specific cytotoxic

T-lymphocyte (CTL) clones are more efficient than those specific for RT(476-484) in lysing infected target cells, because of a better presentation of p17 epitope (35, 38). To understand the molecular basis of the dominance of p17(77-85) over RT(476-484), a detailed analysis of antigen presentation was performed. First, the kinetics and efficiency of the degradation by enriched proteasome fractions of protein fragments encompassing the epitopes were compared. Binding assays of the digestion products to TAP and of the optimal epitopes to HLA-A2 molecules were then performed. The avidities of spontaneously induced CD8⁺ T cells against each epitope in HIV-infected asymptomatic patients were measured.

MATERIALS AND METHODS

Peptides. The synthetic peptide sequences for p17(68-93) (LQTGSEE LRSLYNTVATLYCVHQRIE) and RT(468-495) (LELAENREILKEPVH GVYYDPSKDLIAE) were selected from the p17 and RT HIV type 1 (HIV-1)/Bru isolate and synthesized by Neosystem (Strasbourg, France). Lyophilized peptides were diluted to 1 mg/ml in water plus 10% dimethyl sulfoxide (DMSO), aliquoted, and stored at –20°C.

Cell extract. T1 cells (5×10^8) were lysed in 5 ml of 50 mM Tris (pH 7.5) by Dounce homogenization. After centrifugation (30 min, $17,000 \times g$, 4°C), supernatant was collected and proteins were precipitated with 5% polyethylene glycol 8000 (Sigma-Aldrich, St. Quentin Fallavier, France). After a similar centrifugation, proteins remaining in the supernatant were precipitated with 12% polyethylene glycol 8000 and centrifuged again. Pellet was diluted in 1 ml of 10 mM Tris-HCl–1 mM EDTA–60 μ M ATP (Sigma-Aldrich). Protein concentration was determined (Micro bicinchoninic acid kit; Pierce, Rockford, Ill.), and the presence of proteasomes was assessed by Western blotting with monoclonal antibodies (MAbs) directed against low-molecular-weight protein 2 (LMP-2) and LMP-7 β -subunits and a C3 α -subunit (Affiniti Research Products Ltd., Mamhead, United Kingdom).

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Degradation of p17(68-93) and RT(468-495). A total of 200 µg of peptide p17(68-93) was incubated for 4 h with 90 µg of T1 cell extract at 37°C in buffer containing 10 mM Tris-HCl (pH 8) and 1 mM EDTA. Proteins were precipitated in 10% trifluoroacetic acid (TFA) and centrifuged for 10 min at $17,000 \times g$, and the supernatant containing peptides was collected. The protocol was the same for RT(468-495), except 200 µg of peptide was degraded with 360 µg of T1 cell extract for 30 h. A kinetic study was also performed by degrading 100 µg of the peptides with 45 µg of T1 cell extract for varying lengths of time.

Separation by RP-HPLC. Peptide digests were separated by reverse-phase high-performance liquid chromatography (RP-HPLC) (PerkinElmer, Norwalk, Conn.) in a C_{18} column (Macherey-Nagel, Hoerd, France). An acetonitrile gradient in water, containing 0.1% TFA, was applied (0% for 5 min, 0 to 10% for 5 min, 10 to 20% for 50 min, 20 to 60% for 30 min, and 60 to 100% for 5 min). The peptide elution profile was obtained at 214 nm with an absorbance detector (Applied Biosystems, Roissy, France). Fractions containing peaks were individually collected and lyophilized.

Lymphocyte donors. Peripheral blood mononuclear cells (PBMCs) from eight HIV-1-seropositive asymptomatic individuals (patients P19, P22, P27, and P36 from Immunoco cohorte and Z44, Z49, Z77, and Z112 from the Cochin hospital) were isolated by density centrifugation (Ficoll-Paque; Pharmacia Biotech AB, Uppsala, Sweden) and used after freezing and thawing. Cohorts were established with the approval of the local ethics committee (Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale, Cochin Hospital), and all participants gave their written informed consent for the constitution of cell banks.

Detection of epitope generation. Thawed PBMCs from patient Z44 were cultured overnight and plated in a 96-well nitrocellulose plate at 7×10^5 cells per well in 100 µl of complete medium (RPMI 1640 supplemented with Glutamax, nonessential amino acids, 1 mM sodium pyruvate, 10 mM HEPES buffer, 100 U of penicillin/ml, 100 µg of streptomycin/ml [Life Technologies, Paisley, United Kingdom], and 10% fetal calf serum [PAN Biotech, Aidenbach, Germany]). Lyophilized fractions containing p17 peptides were dissolved in complete medium, and 100 µl per well was added on cells. For RT fractions, the method was the same, except the P19 cell line directed against RT(476-484) was used at 5,000 cells per well. Enzyme-linked immunospot (ELISpot) for gamma interferon (IFN-γ) was performed as described below after overnight incubation.

Mass spectrometry analysis. Mass analysis was performed on a linear matrix-assisted laser desorption/ionization-time of flight Voyager DE-Pro mass spectrometer (PerSeptive Biosystems). The peptides corresponding to the computed masses were identified with General Protein Mass Analysis for Windows version 4.2 software (Lighthouse Data) with $\pm 0.01\%$ mass accuracy.

Edman's sequencing. TFA-soluble products in crude digests were sequenced by automated Edman degradation with a model 473 protein sequencer (Applied Biosystems, Foster City, Calif.). Concentrations of monorepresented amino acids [G, N, A, H, and I for p17(68-93) and N, R, H, G, D, and S for RT(468-495)] were determined for each cycle of sequencing and allowed definition of position and intensity of cleavage. Percentages of COOH-terminal cleavage of the two epitopes were calculated as follows: $\%p17 \text{ cleavage} = [n\{\text{Tyr}\}_1 - n\{\text{Asn}\}_2] / n\{p17(68-93)\}_{\text{total}}$, and $\%RT \text{ cleavage} = [n\{\text{Tyr}\}_1 - n\{\text{Ser}\}_5] / n\{RT(468-495)\}_{\text{total}}$, where $n\{X\}_y$ is the molar quantity of amino acid X at the yth cycle of Edman degradation.

TAP binding assay. The TAP binding assay was performed as previously described (37). Briefly, microsomes from Sf9 cells infected with a *tap1/tap2* recombinant baculovirus were mixed with a reporter-iodinated peptide (R9L) and epitopes or NH₂-terminally extended peptides from p17 and RT were added at increasing concentrations as competitor peptides. The radioactivity bound on microsomes was measured, and 50% inhibition dose (ID₅₀) values were graphically determined for each peptide at the intersection between the dilution curve and the ID₅₀ line (ID₅₀ = 0.525 × binding R9L without competitor).

HLA molecule purification. HLA molecules were purified from Epstein-Barr virus-transformed B-cell line JESTHOM (HLA-A 0201/0201; 12th International Histocompatibility Workshop, Cell Lines Panel) as described in reference 7. Then, 2 µg of exogenous β_2m /ml and 6 mM 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (Sigma-Aldrich) were added just before the addition of exogenous peptide.

HLA-A2-peptide binding and stability. Aliquots of HLA-A2 heavy chains were incubated with different concentrations (10^{-5} to 10^{-8} M) of exogenous peptides and β_2m as reported in reference 7. Correctly folded HLA complexes were detected with M28, an anti- β_2m MAb coupled to alkaline phosphatase, with 4-methyl-umbelliferyl phosphate (Sigma-Aldrich) as substrate. Fluorescence was excited at 360 nm and measured at 460 nm in the Microfluor reader VICTOR (EG & G Wallac, Turku, Finland). For studies on the stability of the complexes, incubation times at 37°C varied from 0 to 24 h.

ELISpot assay. Ninety-six-well nitrocellulose plates (Millipore, Bedford, Mass.) were coated with 10 µg of mouse MAb anti-human IFN-γ (Mabtech, Nacka, Sweden)/ml. Thawed PBMCs from six HLA-A2 asymptomatic patients were plated in triplicate at 10^5 cells per well. Synthetic peptide p17(77-85) or RT(476-484) was then added in dilution from 10^{-5} to 10^{-12} M, and the plates were incubated for 20 h at 37°C in 5% CO₂. Wells were washed extensively between each step and incubated for 1 h with 100 µl of biotinylated mouse MAb anti-human IFN-γ at 1 µg/ml (Mabtech) and then with ExtrAvidin alkaline phosphatase diluted 1/6,000 (Sigma-Aldrich) for 45 min. Spots were developed by adding the chromogenic alkaline phosphatase substrate nitroblue tetrazolium-BCIP (5-bromo-4-chloro-3-indolylphosphate; Bio-Rad, Hercules, Calif.) and counted with a KS-ELISpot automated device (Zeiss, Hallbergmoos, Germany). Concentrations of peptides that gave the half-maximal number of specific IFN-γ-secreting cells were graphically determined.

RESULTS

Degradation of large peptides p17(68-93) and RT(468-495).

Proteasomes have been implicated in the processing of RT(476-484), but their involvement in the generation of p17(77-85) is not clear (33). Lactacystin does not inhibit the production of p17(77-85) in vivo, but the fact that the lactacystin-independent activity of the proteasome may be responsible for p17 cleavage cannot be excluded. Moreover, Gag polyprotein translation generates defective ribosomal products that are specifically degraded by proteasomes and thought to be a source of epitopes (31). To investigate their sensitivity to proteasomal cleavage, synthetic p17(68-93) and RT(468-495), containing epitopes p17(77-85) and RT(476-484) plus 8 to 10 N-terminal and C-terminal flanking residues, were incubated for various times with proteolytic extracts from T1 cells containing IFN-γ-inducible LMP-2 and LMP-7 proteasome subunits. Peptide p17(68-93) was sensitive to degradation mediated by T1 cell extract as shown on RP-HPLC profiles (Fig. 1): numerous products were observed after a 4-h digestion, and accumulation remained after a 20-h digestion. By contrast, RT(468-495) digestion liberated few products that appeared as discrete absorbance peaks after a 20-h digestion (Fig. 1) and the major part of the RT(468-495) peptide, eluting at 52 min, was still present. To determine the presence of epitopes or precursor peptides, samples containing peaks were individually collected and tested by the ELISpot assay. PBMCs from patient Z44, which specifically recognized epitope p17(77-85), were reactive against fractions 5, 10, and 11 after a 4-h degradation of p17(68-93) (Fig. 2). A CD8⁺-T-cell line derived from patient P19 directed against RT(476-484) did not recognize any fraction after a 4-h digestion of RT(468-495) (data not shown). However, after a 30-h digestion, this specific T-cell line recognized peptides from peaks 7 and 8 and, to a lesser extent, peak 6 of the RT digest. All the products in these fractions were analyzed by mass spectrometry, and sequences were deduced from measured masses (Table 1). Only NH₂-terminally extended precursors from the p17 epitope were generated, whereas the RT epitope was generated under both its optimal and NH₂-terminally extended forms. Remarkably, no peptides with correct COOH-terminal cleavage were found in fraction 6 of the RT(468-495) digest. The sensitivity of the mass spectrometer was certainly lower than the recognition by specific T cells; otherwise, the ionization ability of the reactive peptides was too weak and would have limited their detection.

On a bulk of the large peptide digests, Edman sequencing analysis was performed (Fig. 3) and the quantity of mono-

FIG. 1. Kinetics of degradation of p17(68-93) and RT(468-495) peptides by T1 cell extract. One hundred micrograms of peptides was incubated with 45 μ g of T1 cell extract for 4 or 20 h. At $t = 0$, 30 μ g of peptide without cell extract was directly separated by HPLC. Chromatograms obtained are representative of p17 (top) and RT (bottom). Asterisks indicate retention times of undergraded large peptides. Zoomed regions show epitopes or precursor peptides containing peaks (cf. Fig. 2).

represented amino acids [G, N, A, and H for p17(68-93) and N, R, H, and G for RT(468-495)] was determined for each reaction cycle. This method allowed for the definition of major cleavage sites in a peptide sequence (24). The cleavage of p17(68-93) between residues Leu⁸⁵ and Tyr⁸⁶ that could liberate the precursor peptides of the p17 epitope appeared frequently after a 4-h digestion (25% of the total p17 sequences). Cleavage between Val⁴⁸⁴ and Tyr⁴⁸⁵, generating the epitope RT(476-484), was less frequent (6.9%), even with four times more T1-cell proteolytic extract and after a 30-h degradation (see Materials and Methods for percentage calculations). Therefore, both the kinetics of degradation and COOH-terminal cleavage favored p17 epitope precursor production.

TAP transport of NH₂-terminally extended precursors and epitopes. The p17(77-85) and RT(476-484) epitopes have TAP-dependent processing since presentation of these epitopes is not achieved in TAP-deficient cells (35). To compare the efficiencies of binding to the TAPs of these epitopes in the ER, we used panels of NH₂-terminally extended epitopes from 10- to 15-mers with a size compatible with TAP translocation (22). Binding to microsomes from TAP-infected insect cells was determined in a competition assay with a reference peptide (Table 2). Nine- to 12-mer-NH₂-terminally extended p17 peptides showed better binding to TAP than RT peptides. In particular, 11- and 12-mers were more efficient in TAP binding than the epitope RT(476-484). After digestion by T1 cell extract, the generated p17 peptides were RSLYNT VATL (residues 76 to 85) and GSEELRSLYNTVATL (residues 71 to 85) and had ID₅₀s of 13 and 280, respectively, and RT peptides were ILKEPVHGV (residues 476 to 484) and

REILKEPVHGV (residues 474 to 484) and had ID₅₀s of 175 and 47, respectively. Therefore, p17(76-85), a precursor of the p17 epitope, can bind TAP 3.7 times more efficiently than RT peptides.

HLA-A2 binding of p17 and RT epitopes. We investigated the binding of the two epitopes to purified A2-heavy chains in the presence of exogenous β_2m and the stability of formed complexes (Fig. 4). The binding ability of the two epitopes was equivalent to and weaker than that of the high HLA-A2 binder peptide, matrix protein from residues 58 to 66 [M(58-66)] of influenza virus. In addition, M(58-66)/A2 complexes were very stable (half-life, >24 h), whereas the p17(77-85)/A2 and RT(476-484)/A2 complexes showed reduced stability (half-life, 3.5 h). This stability is, however, compatible with the activation of T cells and triggering of killing and with the induction of specific CD8⁺ T cells (36). Association of the two epitopes with HLA-A2 molecules appeared very similar and probably does not generate an imbalance in the recognition by effector cells.

Avidity of naturally induced effector T cells in asymptomatic patients. We therefore determined the avidity of naturally induced T cells specific for p17(77-85) and RT(476-484) in six HLA-A2⁺ asymptomatic patients who exhibited a response against the two epitopes. The effect of peptide dilution was determined in an ELISpot IFN- γ assay, and concentrations of peptides that gave the half-maximal response were graphically defined (Table 3). The avidity range was around 1 to 0.1 nM of synthetic epitope for the T cells of all patients. No difference between avidity directed to p17(77-85)- and RT(476-484)-specific T cells was observed for any given patient.

FIG. 2. Detection of epitopes and their precursors in T1-cell extract digests. After degradation of p17(68-93) and RT(468-495) by T1-cell extract for varying times (4 and 30 h, respectively), digests were submitted to HPLC separation and samples showing peaks of absorbance were individually collected. Fractions were tested for stimulation of specific T cells recognizing HLA-A2 epitopes p17(77-85) and RT(476-484) (Z44 PBMC and P19 cell lines, respectively). Asterisks indicate fractions inducing IFN- γ secretion. Number of spot-forming cells (SFC) per well is represented.

DISCUSSION

Although p17(77-85) and RT(476-484) are two immuno-dominant HIV epitopes, it has been previously shown that they display disparate antigenicities. Their recognition by specific CTLs on HIV-infected cells correlates to their number of copies per cell [400 for p17(77-85) versus 12 for RT(476-484)]

(35). Several steps of antigen processing are likely to be involved in the different levels of expression of the two epitopes. First, the number of p17 protein precursors is eight times higher than that of RT precursors, which is due to an inefficient

TABLE 1. Identification of p17 and RT peptides recognized by T cells in HPLC fractions				
Peptide and peak	Measured mass (M) ^b	Theoretical mass (M + H) ^c	Sequence ^a	Position
p17				
5	1,137.56	1,136.62	RSLYNTVATL	76–85
10	1,652.79	1,651.84	GSEELRSLYNTVATL	71–85
	1,753.84	1,752.89	TGSEELRSLYNTVATL	70–85
11	1,997.17	1,996.23	LQTGSEELRSLYNTVATL	68–85
RT				
6	No match			
7	991.57	990.59	ILKEPVHGV	476–484
8	1,276.78	1,275.73	REILKEPVHGV	474–484

^a The tolerated gap between the theoretical and measured masses is $\leq 0.01\%$.
^b M, mass of the measured ionized peptide.
^c M + H, theoretical mass of the peptide plus one proton.

FIG. 3. Cleavage sites in the p17(68-93) and RT(468-495) epitopes. After T1-cell extract digestion, cleavage sites were determined by Edman degradation on bulk digests. Sites are indicated by arrows of various thickness proportional to intensity of cleavage.

TABLE 2. TAP binding of p17 and RT epitopes and NH₂-terminally extended precursors

p17 peptide		RT peptide	
Position	ID ₅₀ ^a	Position	ID ₅₀
71–85^b	279.5	471–484	327.7
72–85	62	472–484	>10,000
73–85	85.7	473–484	51.9
74–85	7.9	474–484	47.3
75–85	6.3	475–484	5,133
76–85	12.7	476–484	174.6
77–85	8.1		

^a ID₅₀ is expressed in micromolar (μM).^b Peptides detected in T1-cell extract digests are in boldface characters.

frameshifting during elongation of the RT precursor mRNA (14). Thus, the abundance of proteins, the source of the epitopes, might be the first step in generating an imbalance in epitope presentation. A second critical step is the degradation of the two proteins by cellular enzymatic complexes. We approached this question by studying the degradation of regions containing the two epitopes flanked by 8 to 10 residues: p17(68-93) and RT(468-495). In several reports, degradation of large peptides issued from an antigen allowed researchers to predict epitopes (15, 20). Proteasomal degradation of large peptide Ova(239-281) and of the ovalbumin protein gave the same precursors of the immunodominant epitope Ova(259-267) (2, 3, 26). We also observed that large Nef peptides (residues 66 to 97 and 117 to 145), which are rich in HLA binding motifs and well cleaved by proteasomes, may be generated in vaccinated individuals' T-cell responses directed to the predicted epitopes (6, 11, 29). Here, we showed that complete degradation by T1 cell extract enriched in proteasomes containing LMP-2 and LMP-7 subunits occurs in 8 h for p17(68-93) versus >72 h for RT(468-495). Furthermore, on HPLC gels, peaks containing epitope or precursor peptides appeared as soon as 2 h after p17 degradation whereas 8 h was required for RT degradation (data not shown and Fig. 1). Since generation of epitopes by the proteasome depends on adequate cleavage at their COOH-terminal end (8), the frequency of bond cleavages at this position in large peptides would correlate to the quantity of epitopes liberated. Our analysis revealed a cleavage preference of T1 cell extract for the bond between Leu⁸⁵ and Tyr⁸⁶ in p17 rather than that between Val⁴⁸⁴ and Tyr⁴⁸⁵ in RT (25 and 6.9% of total p17 and RT peptides, respectively). Moreover, the impact of unfolding on proteasome degradation could be important, considering that regions after an α-helix are well unfolded whereas those placed after a β-sheet are inefficiently unraveled (19). Epitope p17(77-85) is located in an α-helix (21), whereas RT(476-484) is contained within a β-sheet (30). This may explain why p17 epitope precursors are generated faster and more frequently than those for the RT epitope. Degradation of RT(468-495) generated the optimal RT epitope and an NH₂-terminally extended precursor from residues 474 to 484, whereas p17(68-93) degradation generated several precursor forms of the naturally processed p17 epitope (76 to 85, 71 to 85, and 70 to 85). The generation of exact epitopes by proteasomes is a matter of some debate (25), and extended epitope precursors have been described previously (4, 6, 16, 18). Generation of precursors

FIG. 4. Association of p17 and RT epitopes with HLA-A2 and stability of formed complexes. (A) Binding to purified HLA-A2 molecules of p17(77-85) and RT(476-484) and to influenza virus epitopes M(58-66), presented by HLA-A2, and NP(383-391), presented by HLA-B27. Results are expressed as the percentage of M(58-66) binding at 1 μM. (B) Stability of complexes at 37°C was determined at different time points after initial association and stabilization of HLA-A2-purified molecules with 1 μM concentrations of each peptide. Results are the means from three experiments.

implies that further trimming should take place before fixation of the peptide on MHC molecules and that three aminopeptidases can remove NH₂-terminal amino acids on precursor peptides in the cytoplasm (1, 34). The generation of optimal p17 epitope by an aminopeptidase present at the outer membrane of the ER was recently described (10). This could ame-

TABLE 3. Avidity of effector cells from HIV-1-positive asymptomatic patients against p17 and RT epitopes

Patient	p17(77–85)		RT(476–484)	
	MR ₅₀ ^a	SFC ^b	MR ₅₀	SFC
P22	0.8	131	1	1,879
P27	2	1,515	1	2,083
P36	0.7	222	1	382
Z49	1	3,884	1	760
Z77	2	1,959	1	1,504
Z112	0.1	136	0.1	206

^a MR₅₀ (concentration of epitope giving the half-maximal T-cell response) was graphically determined and is expressed in nanomolar (nM).

^b The number of spot-forming cells (SFC) is given at a peptide concentration of 1 μM for 10⁶ PBMCs.

liorate presentation of this epitope by a better binding to TAP (Table 2). Other uncharacterized endoplasmic aminopeptidase activities involved in the trimming of precursors (17, 32) were also implicated in the generation of p17 and RT epitopes (10). Since presentation of the p17 and RT epitopes was also dependent on translocation in the ER by TAP (35), we tested TAP binding of these epitopes and their NH₂-terminally extended peptides generated by T1 cell extract. The p17(76-85) epitope precursor binds over 13 times more efficiently than the RT(476-484) epitope and 3.7 times more efficiently than the RT(474-484) epitope precursor. Therefore, competition for entry in the ER presumably favors p17 epitope precursors, since it has been demonstrated that peptide translocation ability in the ER is directly related to TAP binding (13).

It is surprising to note that, despite a great difference in the number of p17 and RT epitopes presented on infected cells, specific T-cell responses were of similar avidity and of similar frequency in HLA-A2⁺ HIV-infected subjects, both in the acute phase (2 of 13 versus 1 of 13, respectively) and the asymptomatic phase (10 of 14 for the two epitopes) (9). Several hypotheses could account for this observation. (i) Chronical antigenic restimulation and high HIV antigen load could allow an expansion of RT-specific T cells as important as p17-specific T cells, which would saturate the induction capacity of CD8⁺ T cells. This would cause differences in the kinetic aspect of induction, but this has not been observed. (ii) The high number of total peptide/HLA-A2 complexes at the surface of professional antigen-presenting cell (pAPC) implies a large enhancement of RT(476-484) presentation but also a conservation of dominant p17(77-85) presentation. Since the number of antigenic complexes per pAPC is correlated to the number of circulating precursors (12), a predominance of p17(77-85)-specific T cells would be expected, but this was not observed in patients tested (Table 3). (iii) Different antigen-processing pathways in pAPCs and in target cells could generate various amounts of epitopes or precursors. To support this hypothesis, a differential generation of the RU1 epitope by standard proteasomes and immunoproteasomes was recently described (23). Alternatively, a CTL response was induced in TAP-deficient mice against the dominant Ova(259-267) epitope but was strictly TAP dependent when introduced as a minigene in cells (27). TAP-dependent and -independent pathways have been described for cross-presentation of heat shock protein-linked peptides in macrophages (5) and could support this hypothesis.

By an extensive analysis of antigen processing, our study demonstrates that several steps are implicated in the imbalance of p17(77-85) and RT(476-484) presentation on infected cells. Our results are coherent with the differences observed in the antigenicity of the two epitopes. By contrast, T-cell responses of infected patients reveal that other unexplained mechanisms are involved in the similar immunogenicities of these epitopes.

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