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Selection of T Cell Clones Expressing High-Affinity Public TCRs within Human Cytomegalovirus-Specific CD8 T Cell Responses[1]

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Assessment of clonal diversity of T cell responses against human CMV (HCMV), a major cause of morbidity in immunodepressed patients, provides important insights into the molecular basis of T cell immunodominance, and has also clinical implications for the immunomonitoring and immunotherapy of HCMV infections. We performed an in-depth molecular and functional characterization of CD8 T cells directed against an immunodominant HLA-A2-restricted epitope derived from HCMV protein pp65 (NLV/A2) in steady state and pathological situations associated with HCMV reactivation. NLV/A2-specific T cells in healthy HCMV-seropositive donors showed limited clonal diversity and usage of a restricted set of TCR Vβ regions. Although TCRβ-chain junctional sequences were highly diverse, a large fraction of NLV/A2-specific T cells derived from distinct individuals showed several recurrent (so-called "public") TCR features associated in some cases with full conservation of the TCRα chain junctional region. A dramatic clonal focusing of NLV/A2-specific T cells was observed in situations of HCMV reactivation and/or chronic inflammation, which resulted in selection of a single clonotype displaying similar public TCR features in several patients. In most instances the NLV/A2-specific dominant clonotypes showed higher affinity for their Ag than subdominant ones, thus suggesting that TCR affinity/avidity is the primary driving force underlying repertoire focusing along chronic antigenic stimulation. *The Journal of Immunology,* **2005, 175: 6123–6132.**

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Human CMV (HCMV)[3] is a ubiquitous β-herpesvirus that infects between 60 and 90% of individuals, depending on the population studied. This virus persists lifelong in a latent stage after primary infection, but can be reactivated in situations of immunodepression and/or chronic inflammation (1). HCMV infections are usually asymptomatic in immunocompetent individuals, but can cause serious disease in immunologically immature and immunocompromised individuals (2, 3). Direct contribution of CD8 T cells to the immunological control of HCMV infections is suggested by adoptive transfer clinical trials performed with HCMV-specific CD8 T cell clones (4). Such trials also suggest that CD4 T cells are needed to sustain long-term immunity against HCMV (for a recent review, see Gandhi et al., Ref. 5).

Dominant HCMV-specific CD4 and CD8 T cell responses are primarily directed against two HCMV proteins, namely the viral tegument protein pp65 and the immediate early gene product IE-1 (5–7). Within these proteins, particular epitopes are preferentially targeted by CD4 and CD8 T cells in individuals sharing a given *HLA* allele. For instance, the vast majority of pp65-specific CD8 T cells in *HLA-A2*$^+$ individuals recognizes the same HLA-A2-restricted epitope (pp65$_{495–503}$, hereafter referred to as NLV/A2) (6, 8–10). Such NLV/A2-specific T cells represent up to several percent of the peripheral CD8 lymphoid pool in kidney or bone marrow-transplanted patients undergoing HCMV reactivation (11). Moreover, these dominant HCMV-specific CD4 and CD8 T cell responses are highly oligoclonal, as indicated by flow cytometric and molecular analyses of their TCR repertoire (6, 8–10). Therefore, it seems that efficient control of HCMV infections can be achieved by a few CD8 and CD4 clones directed against a limited set of epitopes.

The specific contribution of T cell subsets directed against a given HCMV epitope to the overall immune protection and the size of the necessary and sufficient T cell repertoire ensuring long-term protective immunity against HCMV remain debated. Adoptive transfer experiments suggest that a single CD8 T cell clone directed against the dominant NLV/A2 epitope can provide at least transient protection against HCMV infections in susceptible recipients (4, 12). However, the respective protective efficacy of T cell clones showing different specificities is yet unclear. Besides, even within NLV/A2 specific T cells, the influence of particular functional parameters, such as the functional affinity/avidity for Ag, on T cell clone protective efficacy in an adoptive transfer setting remains unknown.

Another important and unresolved issue deals with the mechanisms underlying immunodominance of T cell responses directed against particular HCMV epitopes. This issue has been previously addressed in other infectious and noninfectious contexts through combined analyses of the frequency within the naive repertoire, the clonal diversity (estimated by TCR repertoire analysis), and the

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[3] Abbreviations used in this paper: HCMV, human CMV; pMHC, peptide/MHC; HD, healthy donor, SF, synovial fluid lymphocyte; RA, rheumatoid arthritis; KT, kidney transplant; BMT, bone marrow transplant; GVHD, graft-vs-host disease; BLC, B lymphoblastoid cell.

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TCR affinity/avidity of T cells directed against defined peptide/ MHCs (pMHC; Refs. 13–17). So far, such studies have yielded contrasted results, documenting in some but not all instances correlation(s) between immunodominance and one or several of the above parameters. A highly restricted TCR repertoire of Ag-specific T cells associated with biased usage of particular TCR Vα or Vβ regions and constraints on the length and/or amino acid sequence of the TCR CDR3 loops (i.e., the VJα and VDJβ regions) have been reported in some systems (13–20). In a few cases, fully conserved (so-called "public") CDR3 were identified within clonotypes derived from distinct individuals (15–20). Such public TCR were expressed by clonotypes chronically exposed to their selecting Ag and displaying similar TCR affinities (16, 17), suggesting that CDR3 restriction resulted from TCR affinity focusing. On a clinical standpoint, identification of such public TCR motifs have proven particularly useful to design TCR clonotypic probes for the molecular follow-up of T cell responses of defined specificity (20). With respect to HCMV-specific responses, TCR repertoire focusing and marked oligoclonality of several dominant HCMV-specific CD4 and CD8 T cell responses have been previously reported (6, 8–10, 21). However, public TCR motifs have not been described so far. Because in most instances, TCR repertoire studies were not coupled to detailed analyses of T cell functional responses, the mechanisms underlying clonal dominance within HCMV-specific T cells remain elusive.

The aim of this study was to assess the clonal composition and TCR affinity/avidity of NLV/A2-specific CD8 T cells, whose immunodominance and contribution to HCMV protective immunity is supported by previous observations (4, 5). To assess the effect of chronic antigenic stimulation on the clonal composition and affinity of NLV/A2-specific T cells, we compared the features of NLV/ A2-specific T cells in steady state vs pathological situations associated with HCMV reactivation (i.e., in immunodepressed graft recipients or patients with chronic inflammatory diseases) (22, 23). Our results suggest that the NLV/A2-specific T cell response is far more constrained than initially assumed, and is composed of several common clonotypes harboring public TCR features. A dramatic clonal focusing of NLV/A2-specific T cell responses occurred in situations of immunodepression and/or chronic inflammation. Reduction of clonal diversity was associated with selection of clones displaying high-affinity TCR with the same public motifs in several donors. Such observations have both fundamental and clinical applications for our understanding of HCMV clonal dominance and for the rational design of immunotherapeutic approaches targeting this virus.

Materials and Methods

Generation of recombinant pMHCs

Soluble pMHC monomer NLV/A2 was generated as described (24). In brief, β2-microglobulin and HLA-A2 H chain were produced as inclusion bodies in *Escherichia coli*, dissolved in 8 M urea, and refolded in the presence of 15 μg/ml of the pp65 synthetic peptide NLVPMVATV (Genosys). The folded pMHC complexes were diafiltrated and concentrated in 10 mM Tris (pH 8) on a prep scale 3-kDa concentration cassette (Millipore). pMHCs were biotinylated using BirA enzyme, purified by anion exchange on a MonoQ column (Pharmacia) with a 0–0.5 M NaCl gradient, and checked by gel filtration on a Superdex 200 column (Pharmacia). For flow cytometry analyses, pMHC monomers were tetramerized with PE-labeled streptavidin (Sigma-Aldrich) at a molar ratio of 4:0.8. In most instances, we used HLA-A2/peptide complexes harboring an Ala to Val substitution at position 228 of the HLA-A2 H chain: such mutated pMHC show reduced affinity for CD8 coreceptor and are suitable for immunomagnetic sorting of Ag-specific T cells (25) and for staining analysis of both CD8-dependent and -independent T cells. We also made HLA-A2/ peptide complexes in which the α3 domain of the HLA-A2 H chain was replaced by an α3 domain from a murine *H-2* allele (A2/K^b tetramers). To abolish the CD8 binding site on the A2/K^b tetramers and overcome the high affinity interaction of human CD8 with A2/K^b tetramers, we engineered a mutant of A2/K^b tetramers, in which residues Gln^{226} and Asp^{227} were mutated to Ala and Lys, respectively. Such pMHC (hereafter referred to as CD8-null tetramers) are no longer able to bind to human CD8 coreceptors and can be used to identify T cell subsets bearing high-affinity TCR (26).

Generation of T cell clones

T cell lines were derived from either PBL of *HLA-A2* CMV-seropositive healthy donors (HD), synovial fluid lymphocytes (SF), or PBL from *HLA-A2* rheumatoid arthritis (RA) patients, PBL from *HLA-A2* kidney transplant (KT) patients or PBL from one HLA-A2 bone marrow transplant (BMT)-grafted patient undergoing acute HCMV reactivation. The clinical status of RA patients has been detailed elsewhere (27, 28). KT1, KT2, KT4, KT5, KT6, and KT7 patients corresponded to CMV$^-$ recipients receiving a CMV$^+$ kidney graft, whereas in the case of KT3, both graft recipient and donor were CMV$^+$. In all instances, KT PBL samples were drawn from patients with positive pp65 antigenemia. T cells were cultured, expanded, and cloned in RPMI 1640 supplemented with human serum 8% and rIL-2 (150 IU/ml) and were stimulated once a month with purified leukoagglutinin at 1 μg/ml, irradiated PBL, and B lymphoblastoid cells (BLC) (29).

Sorting of Ag-specific T cells using recombinant pMHC NLV/A2 was performed on CD8$^+$ sorted T lymphocytes as previously described (25). In brief, streptavidin-coated immunomagnetic beads (Dynabeads M-280 streptavidin; Dynal) were incubated for 1 h with biotinylated pMHC monomers (1 μg of monomer for 10 μl of beads) at room temperature, washed once, and added to 10^7 CD8$^+$ T cells in 700 μl of PBS-BSA 0.1%. Cells were rotated 4 h at room temperature, washed eight times as previously described (25) and expanded as described above. The purity of Ag-specific T cells was checked by tetramer staining and two successive sortings were made, when necessary, to achieve a purity of >96%. T cell-specific populations were then cloned and stimulated as previously described (29).

Flow cytometric analysis

T cell lines and T cell clones were phenotyped by indirect immunofluorescence using mAb against TCR Vβ regions (30, 31). Stainings with NLV/A2 tetramer were performed by incubating cells for 1 h at room temperature with PE-labeled pMHC tetramer at 20 μg/ml in PBS-BSA 0.1%. After staining, cells were analyzed by flow cytometry on a FACScan apparatus using CellQuest software (BD Biosciences).

Functional assays

pp65 synthetic peptide was dissolved at 20 μg/ml in DMSO, diluted at 2 mg/ml in 10 mM acetic acid, and then diluted at the requested concentration in RPMI 1640 10% FCS. Cytotoxicity was measured in a standard 4-h ^{51}Cr release assay (29). Briefly, BLC were labeled with 100 μCi of $Na_2{}^{51}CrO_4$ (Oris Industries) for 1 h at 37°C, washed three times in RPMI 1640 10% FCS, and pulsed with various concentrations of peptide for 1 h at 37°C. After two washes, target cells were incubated with the different T cell clones at an E:T ratio of 10:1 in 150 μl of RPMI 1640 10% FCS for 4 h at 37°C. Supernatant (25 μl) from each well was removed and counted in a gamma scintillation counter.

For CD8 blocking experiments, T cells were incubated with the anti-CD8 mAb B9–11 (Immunotech) at 10 μg/ml for 15 min before incubation with target cells (32).

pMHC tetramer dissociation assay

T cell clones were stained with NLV/A2 tetramer for 1 h at room temperature with PE-labeled pMHC tetramer at 20 μg/ml in PBS-BSA 0.1%. Cells were washed three times and resuspended in 100 μl/well of PBS-BSA 0.1% containing BB7.2 anti-MHC blocking Ab at 100 μg/ml in the number of wells needed for the different time points. At appropriate time points, cells were removed, washed twice in PBS-BSA 0.1%, and fixed in 2% paraformaldehyde for further analysis by flow cytometry (33).

Sequence analysis of TCR transcripts

RNA from 5×10^6 T cell clones was extracted with TRIZol reagent (Invitrogen Life Technologies). Reverse transcriptions and PCR amplifications were performed as described (29–31). Sequences were conducted on purified PCR products with the ABIPRISM Big Dye Sequencing kit (Applied Biosystems) according to supplier's instructions. Sequence products were then migrated in a 377 DNA sequencer and analyzed with the Sequencing Analysis software (Applied Biosystems). Throughout the manuscript, we have followed the TCR nomenclature of Arden et al. (34).

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Results

Restricted TCR usage by T cells directed against NLV/A2

Although the TCR features of NLV/A2-specific T cells were previously described by several groups, such studies were performed on a rather limited number of clones and donors, and focused on the analysis of TCRβ chain features only (6, 8–10). The features of NLV/A2-specific T cells selected in pathological situations associated with HCMV reactivation have not yet been assessed. In the present study, we undertook a detailed analysis of the TCRα and TCRβ repertoire of NLV/A2-specific T cells derived from both HCMV-seropositive HDs and patients under chronic immunosuppression (i.e., in RA and kidney allograft (KT) recipients) as well as in a BMT recipient under acute HCMV reactivation. To this end, we derived NLV/A2-specific T cells from patients and several HDs after sorting with immunomagnetic beads coated with mutated HLA-A2/peptide complexes showing reduced affinity for CD8 coreceptors. Such an approach allows efficient isolation of Ag-specific T cell lines whose phenotypic and molecular features can then be studied (20, 25, 31).

The TCR repertoire and clonality of NLV/A2-specific T cells was first analyzed by immunofluorescence using anti-Vβ-specific Abs (Table I). In agreement with previous studies, NLV/A2-specific T cells derived from HDs used a limited but heterogeneous set of TCR Vβ regions because at least seven distinct Vβ subsets were identified within these cell lines. Four TCR Vβ regions (BV8S1, BV13S1, BV14S1, and BV20S1) were recurrently used by at least 3 of 5 donors, which implied the existence of clonotypes sharing recurrent TCR features within the NLV/A2-specific T cell immune response. NLV/A2-specific T cells derived from BMT, RA, or KT patients showed an even more dramatic skewing of their Vβ repertoire, as in most patients, >95% of specific T cells derived from a given donor expressed the same Vβ region (Table I). Dominant subsets from distinct patients frequently used the same Vβ, such as BV13, which was expressed by 75–100% of NLV/A2-specific T cells in 7 of 15 individuals. In an attempt to correlate TCR Vβ diversity and frequency of NLV/A2-specific T cells, we estimated the percentage of NLV/A2$^+$ T cells within unsorted CD8 T cells from three HDs (PBL2, PBL3, PBL5), five RA patients (RA3, RA5, RA11, RA14, RA15), and four KT patients (KT1, KT2, KT3, KT5). Although in HDs and several patients the frequency of NLV/A2$^+$ T cells was too low (i.e., below 0.10%) to permit accurate analysis of their Vβ repertoire, the four patients whose frequencies of NLV/A2$^+$ T cells were above 0.1% showed a highly homogeneous NLV/A2$^+$ TCR Vβ repertoire (Fig. 1 and data not shown). In particular, most NLV/A2$^+$ T cells from patients RA11, RA14, KT1, and KT3–which represented, respectively, 2.22, 0.12, 0.97, and 0.13% of CD8 T cells– expressed the same Vβ region, which corresponded to the dominant one identified within expanded NLV/A2$^+$ cells (i.e., Vβ13 for RA14 and KT1, Vβ14 for RA11, and Vβ3 for KT3; Fig. 1 and Table I). Therefore, these results strongly suggest that the TCR Vβ repertoire focusing observed within NLV/A2$^+$ T cells derived from several patients is due to prior in vivo T cell clonal expansion rather than an in vitro culture bias.

To study the clonal diversity and TCR junctional features of NLV/A2-specific clones, NLV/A2-specific T cell lines were cloned and their expressed TCR genes were systematically sequenced (Table II). In agreement with previous reports, the CDR3β sequences were highly diverse, even within subsets using the same Vβ region. However, marked interindividual conservation of VJα junctional sequences was noted within subsets expressing identical VαVβ combinations (e.g., AV18S1BV13S1 or AV15S1BV20S1). These public clonotypes with highly conserved CDR3 sequences were found in both HDs and immunodepressed patients. We identified four major public VJα motifs shared by TCR derived from distinct individuals, namely a 6-aa

Table I. *Flow cytometry analysis of NLV/A2 T cell lines using TCR Vβ-specific mAb*[a]

	% of NLV/A2$^+$ T Cells Stained by TCR Vβ-Specific mAb																								
	1	2	3	4	5.1	5.2	5.3	6.2	6.3	6.7	7	8	9	11	12	13.1	13.6	14	16	17	18	20	21	22	23
HD1		6.6										5.2				26		3.4							
HD2																45		14				12			
HD3	13																					63			
HD4		2										81													
HD5												67				1.7		6				1.7			2.3
BMT																98									
RA3 PBL																						99			
RA3 SF																						98			
RA5 PBL																		100*							
RA5 SF																95									
RA11 PBL																		98							
RA11 SF																		99							
RA14 PBL																98									
RA14 SF																95									
RA15 PBL																100*									
RA15 SF																100*									
RA 16									100*																
RA 17												9				43		10				33			
KT1																91									
KT2																97									
KT3			98																						
KT4								100*																	
KT5		4										4				76.6									
KT6	10											12				32									
KT7												22				16		10					5		

[a] The percentage of NLV/A2$^+$ cells stained by mAb specific for particular TCR Vβ regions mentioned on top of the table are indicated. NLV/A2-specific T cell lines from HDs (HD1 to HD5), BMT, and KT recipients were all derived from PBL. Specific T cell lines derived from RA patients were either derived from PBL or SF, at various time points detailed in Table IV. 100*, percentage determined by TCR sequencing.

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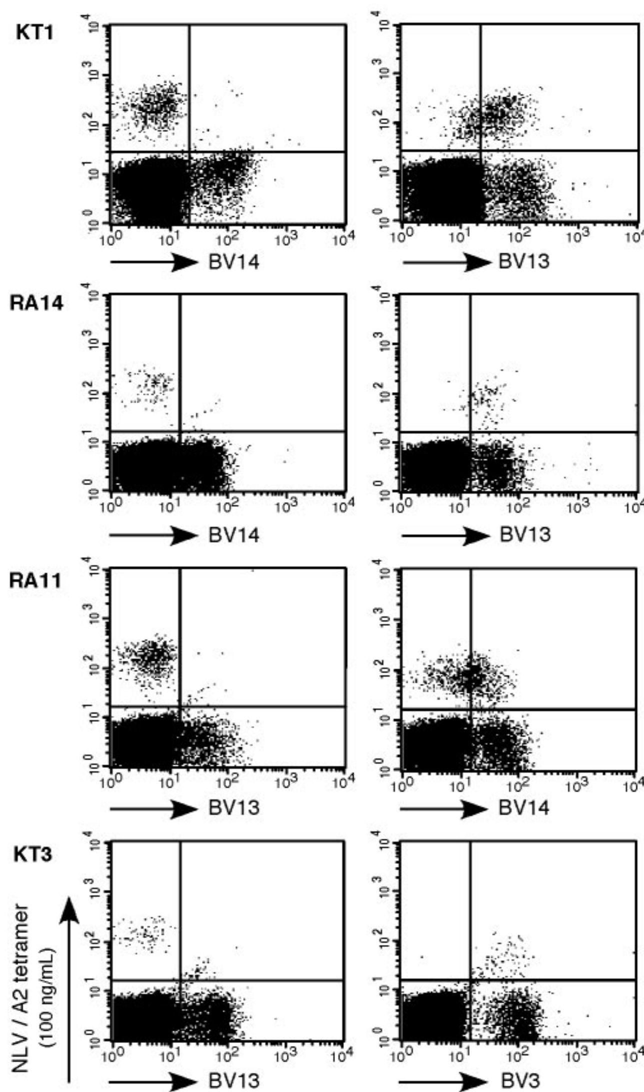

FIGURE 1. Dominant expression of a single Vβ region by unsorted NLV/A2$^+$ T cells from several RA and KT patients. TCR Vβ repertoire of NLV/A2$^+$ T cells within unsorted CD8$^+$ PBL was analyzed by three-color flow cytometry using NLV/A2 tetramers, CD8$^-$, and TCR Vβ-specific mAb. Shown are the fluorescence dot plots of CD8 T cells from two RA and two KT patients whose NLV/A2$^+$ T cell frequencies were above 0.10%, using control (*left dot plots*) and relevant (*right dot plots*) Vβ-specific mAb.

AV18S1AJ49 sequence shared by most BV13 clones and some BV14S1 clonotypes (consensus: xxGNQF), a 7-aa AV18S1AJ43 sequence shared by several BV14S1 clonotypes (consensus: PYNNNDM), an 8-aa AV15S1AJ44 sequence shared by BV20S1 clonotypes (consensus: Y(T)GTASKL), and a 9-aa AV25S1AJ50 sequence (consensus: PxKTSYDKV) shared by BV8S1 clonotypes (Table III). The most frequent public TCRα sequence (AV18S1AJ49) was found in eight distinct individuals (Table II). The canonical motif NTGNQF was also identified in an AV1S4AJ49 rearrangement expressed by a BV13S1 clone (no. R-4, Tables II and III).

TCR repertoire focusing in pathological situations

Although some public clonotypes were detected within NLV/A2-specific T cells derived from HDs, the clonal composition of NLV/A2-specific T cell responses in each individual was quite heterogeneous, as it comprised at least three to nine clones expressing distinct TCR sequences and up to seven distinct Vβ regions (see e.g., HD no. 5 in Tables I and II). By contrast, and in agreement with flow cytometry analysis of the TCR Vβ repertoire (Table I), most NLV/A2-specific clones derived from immunodepressed patients and/or with chronic inflammatory disease expressed the same TCR (Table II and Fig. 2). Consistent with an oligo/monoclonal pattern, direct sequencing of TCR V(D)J junctions performed on several uncloned NLV/A2-specific cell lines yielded readable sequences (data not shown). This was in particular the case for the BMT patient under HCMV reactivation, whose NLV/A2-specific repertoire, which represented up to 15% of its total CD8 PBL pool (data not shown), was composed of a single AV18S1BV13S1 clone (Tables I and II). A monoclonal NLV/A2-specific repertoire was also demonstrated for six of seven RA patients and for four of seven kidney-grafted patients. In six patients, the dominant T cell clone expressed highly related TCR carrying the same public features (Tables II, III, and Fig. 2).

Analysis of T cell samples drawn from the same patient at distinct time points and from distinct tissue locations (e.g., PBL vs synovial fluid in RA patients) showed the presence of the same dominant clone in four of five patients studied (Table IV). For instance in patient RA3, the NLV/A2-specific T cells derived from PBL and SF samples drawn at more than a year interval expressed the same TCR sequences. This strongly suggested that the expanded clonotype(s) in RA patients represented the whole repertoire against the NLV/A2 epitope, which remained stable for at least 1 year.

NLV/A2-specific T cell clones showed similar functional avidities but heterogeneous susceptibility to blocking anti-CD8 mAb

Comparison of the nucleotidic sequences of the public VJα sequences derived from AV18S1BV13S1, AV18S1BV14S1, and AV15S1SBV20S1 clones indicated heterogenous nucleotide sequences due to N diversity, despite conserved amino acid sequences (data not shown). Furthermore, bacterial cloning and large scale sequencing of DNA material amplified from PBL of several CMV-seronegative donors using pairs of AV18S1 and AJ49 primers did not evidence overrepresentation of the most common TCRα public motif (NTGNQF) within bacterial clones carrying 6-aa CDR3 sequences (data not shown). Therefore, the recurrence of public clonotypes could be explained neither by recombination/enzymatic constraints, nor by pre-expansion of particular clonotypes due to, e.g., peripheral homeostatic processes or selection by cross-reactive environmental Ags.

Therefore, we undertook an in-depth functional analysis of NLV/A2-specific T cells to determine whether particular functional features could underly dominance of public clonotypes. Cytolytic activity of NLV/A2-specific CTL was assessed toward HLA-A2 EBV-transformed BLC lines loaded with grading amounts of NLV peptide. Consistent with previous analyses of T cells selected along chronic viral infections (31, 32), NLV/A2-specific T cell clones showed a restricted range of functional avidities despite their different TCR sequences (see representative titration curves in Fig. 3*A*). Peptide concentrations yielding half-maximal lysis of peptide-loaded targets (EC_{50}) ranged from 10^{-7} to 10^{-6} M (Fig. 3*A* and data not shown). As an indirect way to assess TCR affinity, we studied the effect of blocking anti-CD8 mAb on T cell clone responses to grading doses of NLV peptide (31, 32). For CD8 blocking experiments, T cells were preincubated with the anti-CD8 mAb B9–11 prior to the cytolytic assay and CD8 dependence was estimated by calculating the ratio between EC_{50} obtained without and with anti-CD8 mAb (see representative titrative curves in Fig. 3*B* and overall EC_{50} ratios in Table V). CD8

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Table II. *TCRα and β sequences derived from NLV/A2-specific T cell clones*[a]

D	Clone	AV	CDR3 α			AJ	BV	CDR3 β			BJ	f[b]	Public No.[c]	%[d]
HD1	1.1	18S1	CAR	NTGNQF	YFG	49	13S1	CAS	ND	TGF	1S2	1/2	1	26
	1.2	18S1	CAM	NTGNQF	YFG	49	13S1	CAS	ND	TGF	1S2	1/2	1	
HD2	2.0	18S1	CAR	NTGNQF	YFG	49	13S1	CAS	SYVTGTGSYGY	TGF	1S2	*w*	1	45
	2.1	18S1	CAY	PYNNNDM	RFG	43	14S1	CAS	SLEGYTEA	FFG	1S1	4/4	2	14
HD3	3.1	15S1	CAE	YTGTASKL	TFG	44	20S1	CAC	SLRSQGTDTQ	YFG	2S3	3/3	3	63
	3.21	27S1	CAV	DIETSGSRL	TFG	58	IS1	CAS	SELGGAGTGEL	FFG	2S2	1/1		
	3.5	16S1	CAV	YFGNVL	HCG	35	3S1	CAS	SFLQVTEA	FFG	1S1	2/2		
HD4	4.5	25S1	CAG	PRQTSYDKV	IFG	50	8S1	CAS	SSANYGY	TFG	1S2	1/4	4	81
	4.6	25S1	CAG	PRKTSYDKV	IFG	50	8S1	CAS	SSANYGY	TFG	1S2	1/4	4	
	4.7	25S1	CAG	PMKTSYDKV	IFG	50	8S1	CAS	SSAHYGY	TFG	1S2	1/4	4	
	4.8	25S1	CAG	PEKTSYDKV	IFG	50	8S1	CAS	SSANYGY	TFG	1S2	1/4	4	
	4.2	23S1	CAV	YGTASKL	TFG	44	2S1	CSA	FSGAAFVTDTQ	YFG	2S3	2/2		2
	4.1	15S1	CAE	ISPSGGSYIP	TFG	6	15S1	CAT	SDLKVHSGNYGY	TFG	1S2	2/2		
HD5	5.1	25S1	CAG	PMKTSYDKV	IFG	50	8S1	CAS	SSAFYGY	TFG	1S2	1/2	4	67
	5.2	2S2	CAT	KNTGNQF	YFG	49	8S1	CAS	SVGLPYEQ	YFG	2S7	1/2		
	M1	18S1	CAF	IAGNQF	YFG	49	14S1	CAS	SLFVGGPGNEQ	FFG	2S1	3/5	1d	6
	M2	1S4	CAV	AFGNQF	YFG	49	14S1	CAS	TGTSGALYNEQ	FFG	2S1	2/5	1e	
	5.6	18S1	CAR	NTGNQF	YFG	49	13S1	CAS	SYQTGAGGYGY	TGF	1S2	1/1	1	2
	5.5	15S1	CAE	YTGTASKL	TFG	44	20S1	CAW	SSRDSMTDTQ	YFG	2S3	1/1	3	2
	5.23	15S1	CAE	RLQTGANNL	FFG	36	23S1	CAS	SPPAGSYNEQ	FFG	2S1	1/1		2
	5.2	4S1	CIL	LGTGANNL	FFG	36	7S2	CAS	SPKLQGAWAEKL	FFG	1S4	1/2		
	5.3	6S1	CAM	RVPRAQGGSEKL	VFG	57	7S2	CAS	SQDPFAVLNTEA	FFG	1S1	1/2		
BMT	AD-1	18S1	CAR	NTGNQF	YFG	49	13S1	CAS	SFQTGASYGY	TFG	1S2	4/4	1	100
RA3	PBL	15S1	CAP	YTGTASKL	TFG	44	20S1	CAW	SSRGQMTDTQ	YFG	2S3	*w*	3	99
RA5	LS	18S1	CAK	NTGNQF	YFG	49	13S1	CAS	SPQTGTIYGY	TGF	1S2	*w*	1	95
RA11	PBL	18S1	CAF	PYNNNDM	RFG	43	14S1	CAS	SLEGYTEA	FFG	1S1	*w*	2	98
RA14	PBL	18S1	CAR	NTGNQF	YFG	49	13S1	CAS	SPVTGGIYGY	TGF	1S2	4/4	1	98
RA15	PBL	18S1	CAR	NTGNQF	YFG	49	13S3	CAS	SETGATNYGY	TGF	1S2	*w*	1a	100
RA16	OL-1	4S1	CIL	DNNNDM	RFG	43	6S3	CAS	SLAPGATNEKL	FFG	1S4	3/3		100
RA17	R-4	1S4	CAG	NTGNQF	YFG	49	13S1	CAS	SLQTGATFNYGY	TFG	1S2	*w*	1b	43
	R-22	4S1	CIL	SPNNDM	RFG	43	20S1	CAW	SVSDLAKNIQ	YFG	2S4	*w*		33
	R-2	19S1	CAA	RSNFGNEKL	TFG	48	14S1	CAS	SAWDRSSGANVL	TFG	2S6	*w*		10
KT1	GR1	18S1	CAR	QDGNQF	YFG	49	13S1	CAS	SPVAGGAYNEQ	FFG	2S1	*w*	1c	91
KT2	GR2	1S4	CAV	RIDYKL	SFG	20	13S1	CAS	SYSTGAGETQ	YFG	2S5	*w*		97
KT3	GR3	16S1	CAV	YYGQNF	VFG	26	3S1	CAS	SFQGYTEA	FFG	1S1	*w*		98
KT4	GR4	3S1	CAT	DAPGGYQKV	TFG	3	6S2	CAS	SPWTGTLNTEA	FFG	1S1	*w*		100
		12S1	CAP	PSNTGFQKL	VFG	8								

[a] T cell clones were derived from PBL or SFL of HLA-A2 HCMV-seropositive HD, RA patients, BMT, and KT recipients.
[b] Frequency of T cell clones carrying the same Vβ sequence among clones expressing a given Vβ region. *w*, sequence determined from amplified V(D)J transcripts derived from noncloned NLV/A2 cells sorted with the corresponding Vβ-specific mAb.
[c] Public TCR sequence (see Table III).
[d] Percent of cells expressing the corresponding Vβ region within NLV/A2-specific T cells, estimated by flow cytometry (see Table I). The vertical line means that distinct clonotypes account for the Vβ subsets whose percentage is given in *d*.

dependence was quite variable from one clone to another: although anti-CD8 mAb abrogated the cytolytic responses of some T cell clones (e.g., clone nos. 2.1, 5.2, and RA11), it barely affected the response of other ones (e.g., clone nos. 1.1 or GR3) (Fig. 3*B* and Table V). T cell clones bearing public AV18S1BV13S1 TCR were in most instances CD8-independent: however cytolytic response of a BV13S3 clonotype expressing a highly related TCR (no. LS RA15) was almost abrogated by the anti-CD8 mAb (Table V). The two other public clonotypes AV15S1BV20S1 and AV18S1BV14S1 were, respectively, partly and strongly CD8-dependent. In conclusion, there was no clear-cut correlation between CD8 dependency and expression of a public TCR.

Table III. *Public TCR sequences expressed by NLV/A2-specific T cells*[a]

No.	TCRα	Size (aa)	Consensus VJα			TCRβ	Size (aa)	Consensus VDJβ		
1	AV18S1AJ49	6	CAR	NTGNQF	YFG	BV13S1BJ1S2	10	CAS	S..TG..YGY	TFG
1a	AV18S1AJ49	6	CAR	NTGNQF	YFG	BV13S3BJ1S2	10			
1b	AV1S4AJ49	6	CAR	NTGNQF	YFG	BV13S1BJ1S2	12			
1c	AV18S1AJ49	6	CAR	QDGNQF	YFG	BV13S1BJ2S1	11			
1d	AV18S1AJ49	6	CA.	..GNQF	YFG	BV14S1BJ2S1	11			
1e	AV1S4AJ49	6	CA.	..GNQF	YFG	BV14S1BJ2S1	11			
2	AV18S1AJ43	7	CA.	PYNNNDM	RFG	BV14S1BJ1S1	8	CAS	SLEGYTEA	FFG
3	AV15S1AJ44	8	CA.	YTGTASKL	TFG	BV20S1BJ2S3	10	CA.	S.R.QMTDTQ	YFG
4	AV25S1AJ50	9	CAG	P.KTSYDKV	IFG	BV8S1BJ1S2	7	CAS	SSA.YGY	TFG

[a] The public TCR sequence no. 1 and the highly related clonotype nos. 1a to 1c were detected in 9 of 17 individuals. The public TCR sequence nos. 2, 3, and no. 4 were detected in 2, 3, and 2 distinct individuals respectively (detailed TCR sequences provided in Table II).

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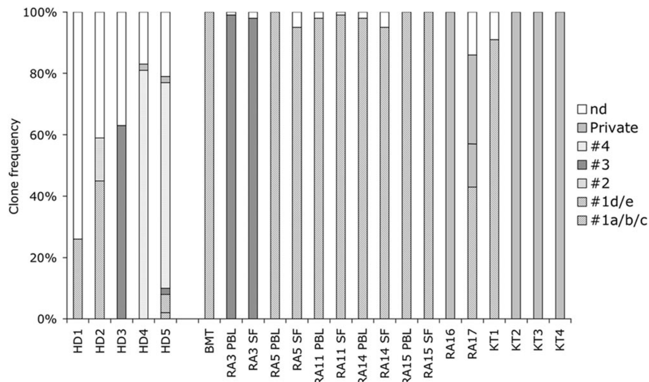

FIGURE 2. Graphical representation of the clonal composition of the NLV/A2-specific TCR repertoire in HDs and patients. The frequency of clones expressing a particular TCR sequences was calculated as described in Tables II-IV.

Nevertheless, clonotypes that expressed similar TCR showed in most instances similar CD8 dependency.

Antigenic affinity and TCR off-rates of NLV/A2-specific T cells correlate with clonal dominance in immunodepressed patients but not with expression of a public TCR

Although there is a gross correlation between resistance to blocking anti-CD8 mAb and TCR affinity, anti-CD8 mAb may in some cases inhibit CD8-independent T cell responses, presumably through delivery of inhibitory signals or nonspecific steric hindrance (35). TCR affinity can be more accurately assessed by analyzing binding of "CD8-null" HLA-A2/peptide tetramers, in which the $\alpha 3$ domain of HLA-A2 has been replaced by the $\alpha 3$ domain derived from a murine *H-2* allele (29). As shown in Fig. 4, some but not all NLV/A2-specific T cell clones were stained by CD8-null tetramers loaded with ad hoc peptide. The most common public clonotypes which predominated in pathological situations associated with recurrent HCMV reactivation (i.e., the AV18S1BV13S1 and AV18S1BV14S1 clonotypes) were stained by CD8 null tetramers, unlike most private clonotypes (Fig. 4 and Table V). However, some less frequent public clonotypes, such as the AV15S1BV20S1 and AV25S1BV8S1 ones, were clearly of low affinity, as indicated by the lack of significant staining with CD8-null tetramers (Fig. 3 and Table II). CD8-null tetramer binding correlated better with clonal dominance than with expression of public TCR: within NLV/A2-specific responses occuring in situations of HCMV reactivation/immune depression, 9 of 11 dominant clones (but 0 of 6 subdominant or minor ones) were stained by CD8-null tetramers in BMT, KT, and RA patients (Table V).

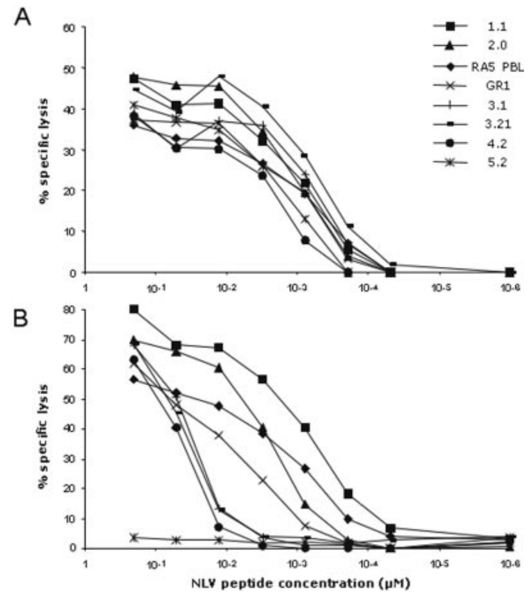

FIGURE 3. Cytolytic responses of NLV/A2-specific clones against peptide Ag-loaded target cells in the absence or presence of blocking anti-CD8 Ab. *A*, CTL dose-response curves (E:T ratio of 10:1) of NLV/A2-specific clones against HLA-A2$^+$ allogeneic B lymphoblastoid cells loaded with various amounts of NLV/A2 peptide. *B*, CTL response of these clones against NLV/A2-loaded targets in the presence of blocking anti-CD8 mAb.

Another parameter that has been previously shown to regulate clonotypic selection in some systems is the overall avidity of the TCR/coreceptor to pMHC complex. Such a parameter can be reliably assessed by measuring the dissociation rate of pMHC tetramers in the presence of a blocking anti-MHC mAb (33). Analysis of NLV/A2 tetramer off-rates in the presence of the HLA-A2-specific mAb BB7.2 led to the identification of two subsets of NLV/A2-reactive T cell clones differing by their dissociation rates (see representative results in Fig. 5). TCR off-rates tightly correlated with CD8-null tetramer binding because of 17 clones for which both parameters were studied, 10 showed fast TCR off-rates and low affinity and 7 showed slow TCR off-rates and high affinity (Fig. 5 and Table V). Altogether these results indicate that 1) TCR off-rate and affinity tightly correlated with the TCR structural features of NLV/A2-specific clonotypes, 2) most but not all public TCR clonotypes expressed high-affinity TCR with slow off-rates, and finally 3) dominant clonotypes identified in immunodepressed patients and/or undergoing HCMV reactivation generally expressed high-affinity TCR with slow off-rates. By contrast there was no clear-cut correlation between clonal dominance and TCR affinity or off-rate in HDs.

Table IV. *TCR sequences of NLV/A2-specific T cell clones derived from distinct tissue locations and/or obtained at different time points*[a]

Donor	Tissue	Date	AV	CDR3 α			AJ	BV	CDR3 β			BJ
RA14	PBL	06–96	18S1	CAR	NTGNQF	YFG	49	13S1	CAS	SPVTGGIYGY	TGF	1S2
	SF	06–96	18S1	CAR	NTGNQF	YFG	49	13S1	CAS	SPVTGGIYGY	TGF	1S2
RA15	PBL	03–97	18S1	CAR	NTGNQF	YFG	49	13S3	CAS	SETGATNYGY	TGF	1S2
	SF	03–97	18S1	CAR	NTGNQF	YFG	49	13S3	CAS	SETGATNYGY	TGF	1S2
RA5	PBL	04–96	4S1	CIL	PLWL	TFG	53	14S1	CAS	SLEGYTEA	FFG	1S1
	SF	04–96	18S1	CAK	NTGNQF	YFG	49	13S1	CAS	SPQTGTIYGY	TGF	1S2
RA11	PBL	06–93	18S1	CAF	PYNNNDM	RFG	43	14S1	CAS	SLEGYTEA	FFG	1S1
	SF	06–93	18S1	CAF	PYNNNDM	RFG	43	14S1	CAS	SLEGYTEA	FFG	1S1
RA3	PBL	06–93	15S1	CAP	YTGTASKL	TFG	44	20S1		ND		
	SF	10–94	15S1	CAP	YTGTASKL	TFG	44	20S1	CAW	SSRGQMTDTQ	YFG	2S3

[a] T cell clones were derived from PBL or SF from RA patients. PBL and SF-derived T cells from RA14 and RA3 patients carried the same VJα rearrangement on the other allele (respectively, AV3S1AJ16 and AV23S1AJ21), thus confirming that they were derived from the same parental cells.

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Table V. *CD8 dependency and TCR affinity of NLV/A2-specific T cell clones derived from HD and patients*[a]

	Dominance	Origin	Clone No.	TCR AV	TCR BV	Public No.	EC_{50}/EC_{50} with Anti-CD8	CD8-Null Binding	Off-Rate
HDs	D	HD3	3.1	15S1	20S1	3	41	−	Fast
	D	HD4	4.7	25S1	8S1	4	19	−	Fast
	D	HD5	5.1	25S1	8S1	4	ND	−	ND
	SD	**HD2**	**2.0**	**18S1**	**13S1**	**1**	**3**	**+**	**Slow**
	SD	**HD1**	**1.1**	**18S1**	**13S1**	**1**	**2**	**+**	**Slow**
	SD	HD2	2.1	18S1	14S1	2	>250	+	ND
	M	HD5	M1	18S1	14S1	1d	ND	+	ND
	M	HD3	3.21	27S1	1S1	−	48	ND	Fast
	M	HD4	4.2	23S1	2S1	−	11	ND	Fast
	M	HD4	4.1	15S1	15S1	−	25	−	Fast
	M	HD1	BV8	ND	8S1	ND	ND	−	Fast
	M	HD5	5.2	4S1	7S2	−	>250	ND	ND
Patients	**D**	**BMT**	**AD-1**	**18S1**	**13S1**	**1**	**13**	**+**	**Slow**
	D	RA3	LS	15S1	20S1	3	18	−	Fast
	D	**RA5**	**PBL**	**18S1**	**13S1**	**1**	**5**	**+**	**ND**
	D	**RA11**	**PBL**	**18S1**	**14S1**	**2**	**>200**	**+**	**Slow**
	D	**RA14**	**PBL**	**18S1**	**13S1**	**1**	**ND**	**+**	**Slow**
	D	**RA15**	**LS**	**18S1**	**13S3**	**1a**	**>150**	**+**	**Slow**
	D	RA16	OL-1	4S1	6S3	−	ND	+	ND
	D	**KT1**	**GR1**	**18S1**	**13S1**	**1c**	**5**	**+**	**ND**
	D	KT2	GR2	4S1	13S1	−	2	−	ND
	D	**KT3**	**GR3**	**16S1**	**3S1**	**−**	**1**	**+**	**ND**
	D	**KT5**	**GR5**	**ND**	**13S1**	**ND**	**ND**	**+**	**Slow**
	SD	RA17	R-22	4S1	20S1	−	ND	−	Fast
	SD	KT6	BV13	ND	13S1	ND	ND	ND	Slow
	M	RA17	R-2	19S1	14S1	−	ND	−	Fast
	M	KT5	BV2	ND	2S1	−	ND	−	ND
	M	KT6	BV8	ND	8S1	ND	ND	−	Fast
	M	KT7	BV13	ND	13S1	ND	ND	−	Fast
	M	KT7	BV8	ND	8S1	ND	ND	−	Fast

[a] CD8 dependency of T cell clones (column EC_{50} ± anti-CD8) was estimated by comparing EC_{50} values obtained in the absence and presence of a blocking anti-CD8 mAb (see representative results in Fig. 3). EC_{50} values correspond to Ag peptide concentration required for half-maximal lysis of peptide-loaded target cells. The affinity of NLV/A2-specific T cell clones was estimated either by flow cytometry analysis of "CD8-null" NLV/A2 tetramer binding (see representative histograms of CD8-null binders and nonbinders in Fig. 4) or by analysis of the kinetics of NLV/A2 tetramer dissociation in the presence of blocking anti-HLA-A2-specific mAb (see representative dissociation curves of clones with fast and slow off-rates in Fig. 5). Note that unlike HDs, most dominant NLV/A2-specific T cell clones (representing above 50% of the NLV/A2-specific repertoire in each individual) expressed high-affinity TCR with slow dissociation rates. Clones with high-affinity TCR and low off-rates are in bold.

Discussion

The present study describes common occurrence of public TCR clonotypes directed against a major immunodominant HCMV epitope, namely NLV/A2. A dramatic clonal focusing of the NLV/A2-specific T cell response was observed in pathological situations associated with either extensive clonal expansion (e.g., in lymphopenic patients undergoing HCMV reactivation) and/or chronic antigenic stimulation (e.g., in patients with chronic inflammatory disease). Such a clonal focusing was associated with overrepresentation of T cell clonotypes displaying high affinity/high avidity for their Ag. These observations have both fundamental and clinical implications that deserve further discussion.

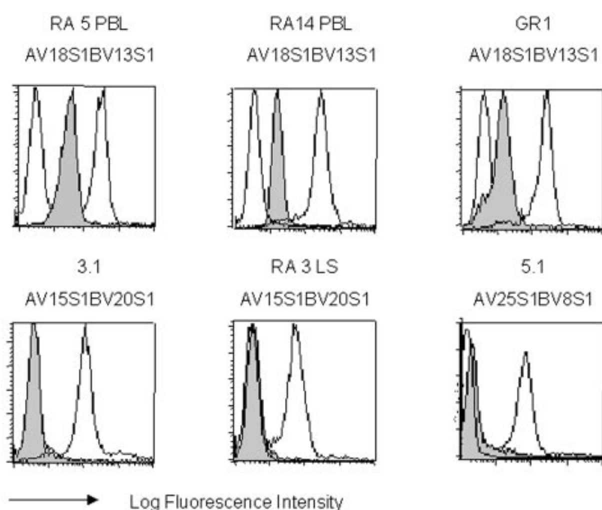

FIGURE 4. Staining of public NLV/A2-specific T cell clones by CD8-null and mutated HLA-A2/peptide tetramers. Representative fluorescence histograms obtained with six clones expressing either AV18S1BV13S1, AV15S1BV20S1, or AV25S1BV8S1 public TCR (see Tables II and III for detailed TCR sequences), using either mutated (white histograms) or CD8-null HLA-A2/NLV tetramers (shaded histograms). Note the significant staining of AV18S1BV13S1 clones (but not the other clones) by CD8-null tetramers.

Identification of NLV/A2-specific clonotypes bearing public TCR was rather unexpected, because several previous studies failed to identify recurrent TCR motifs within T cells showing such a specificity (6, 8–10). The TCRβ chain features of the clones herein described were very similar to those previously reported. In particular our clones used the same set of Vβ genes previously shown to be preferentially expressed by NLV/A2-specific T cells in three other studies (i.e., BV2, 6S1, 6S2, 8S1, 13S1, 14S1, and 20S1) (8–10). The fact that CDR3β sequences were more diverse than CDR3α sequences (Table II), and that previous repertoire analyses of NLV/A2-specific T cell responses focused on the TCRβ chain features expressed by a limited number of clones derived from few donors (8–10), could explain why public TCR motifs have been "missed" so far. Irrespective of this issue, the concordant TCRβ chain features of NLV/A2-specific clones described in the present and previous reports, which were obtained through different selection and culture settings (i.e., after either in vitro peptide specific stimulation or sorting with pMHC multimers), strongly suggest that common occurrence of public clonotypes is a general feature of the NLV/A2-specific response rather

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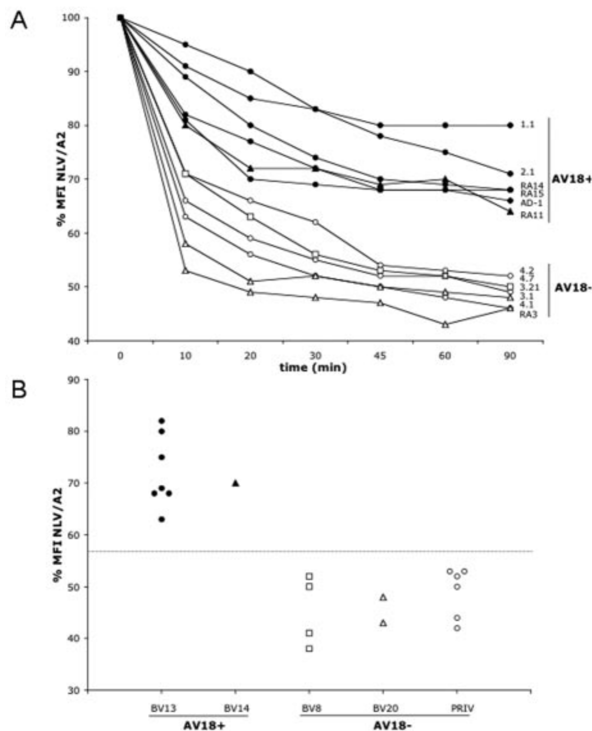

FIGURE 5. Identification of two NLV/A2-specific CD8 T cell subsets differing by their NLV/A2 tetramer dissociation rates. Kinetics of NLV/A2 tetramer dissociation were assessed in the presence of blocking HLA-A2-specific mAb as described in *Materials and Methods*. *A*, Representative dissociation curves obtained with 12 NLV/A2-specific T cell clones. From top to bottom curves: clone nos. 1.1, 2.1, RA14, AD-1, RA15, RA11, 4.2, 4.7, 3.21, 3.1, 4.1, and RA3. *B*, Ratio of NLV/A2 mean fluorescence intensities (MFI) obtained at 60 min of 8 AV18S1$^+$ clones and 12 AV18S1$^-$ clones. From top to bottom values: AV18S1BV13 clones: GR6,1.1, 2.1, RA14, AD-1, RA15, GR5; AV18S1BV14 clone: RA11; BV8 clones: 4.7, HD1BV8, KT6BV8, KT7BV8; AV15S1BV20S1 clones: 3.1 and RA3; private clones: R-2, 4.2, 3.21, R22, and KT7BV13. The dotted line corresponds to an arbitrary threshold between clones with "slow" or "fast" dissociation rates (see Table V), i.e., with values above or below 60%, respectively. Note that all public AV18S1 clones, irrespective of their TCRβ chains, showed slower off-rates than clonotypes expressing either private TCR or public AV18-negative TCR.

than the consequence of an in vitro culture bias specific to this study.

Public clonotypes have been already described in T cell memory responses directed against various Ags but the mechanisms underlying their selection remain debated. Because the canonical sequences of the NLV/A2-reactive T cell clones contained non-germline nucleotides and because the most frequent public AV18S1 TCRα chain sequences were not overrepresented in CMV-seronegative donors, their high frequency could not be accounted for by trivial enzymatic/recombination constraints, by repertoire remolding following thymic selection and/or homeostatic peripheral expansion (36), or by pre-expansion by cross-reactive environmental pathogens (37). Although the most frequent public AV18S1BV13S1 clonotypes expressed TCR with the highest affinity and the slowest off-rates, two other public clonotypes (namely AV15S1BV20S1 and AV25S1BV8S1) were highly CD8-dependent and expressed low-affinity TCR with fast off-rates. Therefore, overrepresentation of public TCR in general does not seem to result from an affinity/avidity-driven selection process, although such a mechanism may favor expansion of clones expressing particular public TCR (such as AV18S1BV13S1 or AV18S1BV14S1) along chronic antigenic stimulation (see below). Recent structural and functional studies suggest that the size of the T cell repertoire (and by inference the extent of structural conservation of TCRs) directed against a given pMHC complex is influenced by the solvent accessibility of the peptidic Ag (38). Although peptides deeply embedded within the MHC groove (also referred to as "featureless" peptides) may select a restricted set of TCR with conserved features, "protruding" peptides may permit selection of a broader (i.e., more diverse) set of TCR (38). The conserved features of NLV/A2-specific TCR suggest that the corresponding pMHC is a featureless one, which might select a rather scarce T cell subset in the naive repertoire. Accordingly, although we could easily sort out naive T cells directed against "bulgy" pMHC such as HLA-A2/Melan-A using ad hoc pMHC multimers (31), all attempts to isolate NLV/A2-specific T cell lines from HCMV-seronegative donors by tetramer-aided immunomagnetic sorting have failed so far (data not shown). This observation, which suggests that immunodominance does not correlate with the frequency of the Ag-specific T cell repertoire in naive individuals, has significant implications for the selection of immunogenic peptides, because protruding but not necessarily dominant HCMV epitopes might be better suited for an efficient generation of HCMV-specific T cells from seronegative donors.

Although oligoclonal, the NLV/A2 repertoire of T cells derived from healthy HCMV-seropositive donors remained quite heterogeneous in a given individual, as in all cases at least 3 and up to 10 distinct specific clones could be derived from each donor. By contrast, the NLV/A2-specific repertoire in the BMT recipient undergoing HCMV reactivation and in most patients with chronic inflammatory disease was composed of a single clonotype. Unlike HDs, the dominant clonotype(s) detected in patients expressed in 9 of 11 cases a high-affinity/avidity TCR, unlike subdominant or minor clonotypes derived from these individuals (Table V). Therefore, clonal focusing in patients is probably due to preferential expansion of high-affinity/avidity clonotypes. Such an affinity/avidity-driven selection process could explain the overrepresentation of the public AV18S1$^+$ clonotypes among the dominant clones found in RA patients and in the BMT recipient under HCMV reactivation. The role of TCR affinity/avidity in peripheral T cell clonal selection is still unclear. McHeyzer-Williams and colleagues (39) recently described the selective loss of clonotypes expressing low-affinity TCR along primary antigenic stimulation, without further selection above this affinity threshold of clones expressing TCR with the highest affinity or half-life. Whether there is further clonal focusing upon secondary/chronic antigenic challenges and the mechanisms underlying this phenomenon are still debated. Many studies have led to opposite conclusions, depending on the model, Ag stimulation modalities, and number of antigenic challenges. However, it seems that stochastic clonotype recruitment and/or lack of clonal focusing is generally observed in situations where the T cell clones showed a narrow range of TCR affinities (40) and/or the Ag was provided in saturating amounts (41). By contrast, when the Ag was provided in limiting amounts or the antigenic selection involved a T cell population with heterogeneous TCR affinities/half-lives, expansion of high-affinity clones seemed to be favored (42, 43). The recurrency of antigenic stimulation and the extent of clonal expansion seem to be additional parameters influencing the clonal composition of the T cell response, particularly in light of modelization studies suggesting that tiny differences in terms of proliferation rate within a population of T cell clones can translate into major differences of clonal frequency after several cycles of cell division. To account for the present and previous findings, a two-step selection process could

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be proposed. Selection of NLV/A2-specific T cells showing similar functional avidities, but heterogeneous TCR affinities and/or half-lives would occur during the primary HCMV response. This could explain the narrow functional avidity window of NLV/A2-reactive T cells derived from both HDs and patients, and the heterogeneous TCR affinities and half-lives of T cell clonotypes in the former case. Such an assumption would be also in agreement with the affinity threshold model proposed by McHeyzer-Williams and colleagues (39). Repeated Ag stimulations (which presumably occur along recurrent HCMV reactivations associated with chronic inflammation), and large clonal expansions associated with HCMV reactivation in a lymphopenic context (in this regard the single AV18S1BV13S1 clone identified in the BMT patient represented up to 15% of its peripheral lymphoid pool), could then favor selection of high-affinity/high-avidity T cell clones. The molecular basis of this affinity-based selection remains unclear: competition for limiting amounts of pMHC complex is one among several possibilities whose testing remains nevertheless beyond the scope of the present study. Finally, the fact that in few patients, the dominant T cell clones did not express high affinity TCR suggests that besides TCR affinity/avidity, other parameters, such as, for instance, the clonal composition of the NLV/A2-specific T cell repertoire before its peripheral selection in the context of HCMV infection, may affect the clonal diversity of the NLV/A2-specific T cell response.

On a more applied standpoint, common occurrence of the highly restricted set of canonical CDR3 sequences listed in Table III, which accounted altogether for 25–80% of the NLV/A2 repertoire of HDs and up to 100% of this repertoire in patients, should allow molecular monitoring of a large fraction of the NLV/A2 repertoire in most HCMV-seropositive individuals using a limited panel of TCR clonotypic probes. Owing to the presumed contribution of NLV/A2-reactive cells to HCMV protective immunity (4, 5), the above approach should permit molecular detection of protective HCMV-specific CD8 T cell responses within biological samples not amenable to functional analyses. Because HCMV T cell responses have been previously implicated in allograft rejection and/or graft-vs-host disease (GVHD) (3), characterization of the alloreactivity patterns of the major NLV/A2-specific public clonotypes could allow analysis of the implication of expanded alloreactive HCMV-specific public clonotype in GVHD or graft rejection processes, through large scale molecular detection of such clonotypes in affected tissues. Along this line, a rational selection of public clonotypes best suited for adoptive immunotherapy could also be foreseen. For instance, the AV18S1BV13S1 public clonotypes, which display high-affinity TCR and presumably limited alloreactivity (owing to their presence in the majority of donors and patients herein studied), could be logical candidates for such a purpose.

Disclosures

The authors have no financial conflict of interest.

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