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HLA B*5701-Positive Long-Term Nonprogressors/Elite Controllers Are Not Distinguished from Progressors by the Clonal Composition of HIV-Specific CD8$^+$ T Cells

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To better understand the qualitative features of effective human immunodeficiency virus (HIV)-specific immunity, we examined the TCR clonal composition of CD8$^+$ T cells recognizing conserved HIV p24-derived epitopes in HLA-B*5701-positive long-term nonprogressors/elite controllers (LTNP/EC) and HLA-matched progressors. Both groups displayed oligoclonal HLA-B5701-restricted p24-specific CD8$^+$ T-cell responses with similar levels of diversity and few public clonotypes. Thus, HIV-specific CD8$^+$ T-cell responses in LTNP/EC are not differentiated from those of progressors on the basis of clonal diversity or TCR sharing.

Understanding natural immunologic control of human immunodeficiency virus (HIV) may provide important insights for the development of vaccines and immunotherapies. Rare long-term nonprogressors/elite controllers (LTNP/EC) maintain durable restriction of HIV replication without antiretroviral therapy (25). Evidence suggesting that HIV-specific CD8$^+$ T cells mediate this control (13) includes a dramatic overrepresentation of the HLA-B*5701 allele (8, 12, 19, 21, 29, 33, 37) and a CD8$^+$ T-cell response focused on HLA-B5701-restricted peptides (11, 29). Similarly, LTNP/EC rhesus macaques are enriched for the Mamu B*08 or B*17 alleles (22, 43) and lose control of simian immunodeficiency virus (SIV) replication following antibody-mediated CD8$^+$ T-cell depletion (9). HIV-specific CD8$^+$ T cells in LTNP/EC also show a greater capacity to suppress HIV replication or kill autologous HIV-infected CD4$^+$ T cells than those of progressors (14, 27, 28, 30, 37). Collectively, these data suggest that greater HIV control in LTNP/EC than in progressors is likely mediated by qualitative differences in their CD8$^+$ T-cell responses.

It has been speculated that favorable HLA molecules may select for a diverse repertoire of T-cell receptor (TCR) clonotypes capable of limiting HIV mutational escape (5, 17, 18, 20, 23, 38). Favorable HLA molecules might preferentially select for "public clonotypes" (10, 34, 38–40), which are shared among multiple individuals and may constitute particularly effective CD8$^+$ T-cell responses (1, 2, 15). In the present study, we compared for the first time the clonalities of CD8$^+$ T cells targeting the same immunodominant HIV epitopes in HLA-B*5701$^+$ LTNP/EC and in progressors to better understand qualitative features of an effective HIV-specific CD8$^+$ T-cell response.

Subjects signed institutional review board-approved informed consent forms and were categorized as LTNP/EC or progressors as previously defined (28, 29). Three progressors who had had elevated HIV RNA levels (>10,000 copies/ml) prior to initiating antiretroviral therapy (ART) donated peripheral blood mononuclear cells (PBMC) while receiving ART yet had persistently detectable viremia due to erratic compliance with their regimens (Table 1). PBMC were stained with HLA-B5701 tetramers complexed to the HIV Gag p24 epitopes ISPRTLNAW (IW9; amino acids [aa] 147 to 155), KAFSPEVIPMF (KF11; aa 162 to 172), or QASQEVKNW (QW9; aa 308 to 316) (Beckman Coulter, San Diego, CA) and then surface stained with antibodies to CD3, CD8, CD57, CD25, CD27, CD28, HLA-DR, CD45RO, CD57, and CCR7 (BD Biosciences, San Jose, CA) prior to flow cytometry analysis (16, 28). Tetramer-positive CD8$^+$ T cells were also sorted at >98% purity by flow cytometry, and all expressed TCRβ gene products were amplified and characterized without bias using a nonnested template-switch-anchored reverse transcription-PCR (RT-PCR) (6, 35). Medians and correlations were analyzed using the Wilcoxon two-sample test and the Spearman rank method, respectively. The Bonferroni method was used to adjust P values for multiple testing. Clonotypic diversity was assessed by the standardized number of clonotypes, which accounts for differences in the number of TCR sequences obtained per sample, and Simpson's diversity index, which accounts for the clonal dominance hierarchy and varies between 0 (minimal diversity) and 1 (maximal diversity) (41).

Between 0.8 and 6.2% of CD8$^+$ T cells from 7 HLA-B*5701-positive LTNP/EC (10 specificities) and 8 progressors (10 specificities) (Table 1) stained positively with at least one HLA-B5701/p24 tetramer (Fig. 1A and B and data not shown). No significant differences were observed between LTNP/EC and progressors within the tetramer-positive populations regarding activation status, costimulation, antigen exposure, or homing (CD25, HLA-DR, CD27, CD28, CD45RO, CD57, or CCR7 expression) ($P > 0.5$ for all comparisons; data not shown). The predominant phenotype of HLA-B5701/p24 tetramer-positive CD8$^+$ T cells was

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TABLE 1 Patient characteristics

Patient type and no.	HLA class I allele(s)			Yr of diagnosis	No. of T cells (cells/μl)		Viral load (copies/ml)	Tetramer(s)[a]
	A	B	C		CD4$^+$	CD8$^+$		
LTNP/EC								
4	1,31	8,57	6,7	1985	943	1,012	<50	IW9, QW9
7	1,2	57	6	1985	325	342	<50	IW9, KF11
33	2,30	13,57	6	1995	1,334	1,557	<50	IW9
34	1,2	8,57	6,7	1989	1,523	1,111	<50	KF11, QW9
30	31,74	51,57	7,16	1990	306	747	<50–58	KF11
6	11,30	52,57	7,12	1986	737	998	<50–152	QW9
8	11,23	44,57	4,6	1985	490	1,033	<50–567	IW9, KF11
Progressors (untreated)								
35	2,3	57	6,7	2000	1,144	1,220	1,800	KF11
20	1	52,57	6,12	1985	1,068	1,091	25,017	KF11
106	3,30	18,57	5,18	1986	420	1,382	24,564	IW9, KF11
131	2,11	35,57	4,6	1989	421	1,082	76,038	IW9, KF11
107	3	40,57	3,7	1987	405	1,418	90,130	IW9, KF11
Progressors (treated)								
105	2,80	8,57	2,7	1990	616	1,026	2,473	KF11
101	1,31	51,57	6,15	1986	419	992	3,421	KF11
103	2,11	55,57	3,6	1991	653	1,130	3,968	KF11

[a] HLA-B5701/HIV Gag p24 tetramers. IW9, ISPRTLNAW (positions 147 to 155); KF11, KAFSPEVIPMF (162 to 172); QW9, QASQEVKNW (308 to 316).

CD25$^-$ CD27$^+$ CD45RO$^+$ CCR7$^-$ CD57$^{+/-}$ in both groups (data not shown), consistent with previous reports (4, 7, 16, 32).

In analyses of the clonotypic composition of HLA-B5701/p24 tetramer-positive cells, a mean of 65 clones were examined for each sorted specificity, representing >1,000 TCR sequences (6, 36). Overall, the immunodominant B5701-restricted HIV p24 epitope-specific CD8$^+$ T-cell populations in both groups were oligoclonal, comprising a median of 6.5 clonotypes (range, 1 to 12) per specificity (Table 2 and Fig. 1A and B). Only the QW9-specific cells of LTNP/EC 4 were monoclonal (Table 2). These data are consistent with previous observations in chronic human viral infections (6, 23).

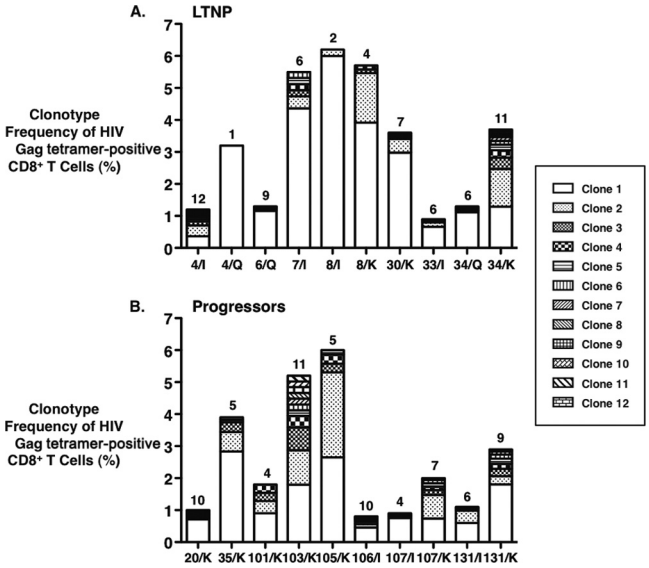

FIG 1 Clonal composition of HLA-B5701/HIV Gag p24 tetramer-positive CD8$^+$ T-cell populations in LTNP/EC and progressors. (A and B) Summary data showing the frequencies of each TCR clonotype (Table 2) relative to the frequencies of the corresponding HLA B5701/HIV Gag p24 tetramer-positive CD8$^+$ T cells in 7 HLA-B*5701-positive LTNP/EC (A) and 8 HLA-B*5701-positive progressors (B). Three HLA-B5701/HIV Gag p24 tetramers containing B57 bound to one of three immunodominant Gag p24-derived epitopes (I, IW9; K, KF11; and Q, QW9) were used to stain and sort antigen-specific CD8$^+$ T cells for clonotypic analysis. Results for two different specificities are shown for patients 4, 8, 34, 107, and 131. The numbers above each stacked bar denote the total number of clonotypes for the depicted epitope-specific CD8$^+$ T-cell population.

To analyze repertoire diversity, subject 107's IW9-specific TCR repertoire (18 TCRs) and subject 101's KF11-specific repertoire (14 TCRs) were excluded due to low numbers of sequences. The remaining data were standardized for sampling differences (41). There were no significant differences between LTNP/EC and progressors in either the standardized numbers of epitope-specific clonotypes per repertoire ($P > 0.5$) (Fig. 2A) or Simpson's diversity index ($P = 0.31$) (Fig. 2C) for the IW9- and KF11-specific repertoires. QW9-specific repertoires were omitted from the comparisons since they were only detected in the LTNP/EC group ($n = 3$) (Fig. 2B and D). Additionally, there were no significant correlations between HIV RNA levels and either the number or diversity of CD8$^+$ T-cell clonotypes per repertoire ($P > 0.5$ for all correlations; data not shown).

We next examined whether there were public TCR clonotypes shared between these patients or with prior reports (10, 38, 44). Only 2 public clonotypes were identified in LTNP/EC that were not shared with patients in this study but that had previously been reported in two progressors (Table 2) (44). In progressors, only two sequences were shared between two individuals and one was previously reported (Table 2) (44). Thus, public clonotypes were not a dominant feature of the repertoires of either LTNP/EC or progressors.

Collectively, these data suggest that the large differences in immunologic control between HLA-B*5701-positive LTNP/EC and progressors are unlikely to be mediated by differences in the TCR

TABLE 2 HLA-B5701-restricted HIV Gag p24-specific CD8$^+$ TCRβ repertoires[a]

Patient	TRBV	CDR3 Sequence	TRBJ	Frequency (%)
4-I	7-8	CASSLRGGGASYNEQF	2-1	30.6
	7-8	CASSPWTYGTGTLNEKLF	1-4	27.8
	11-2	CASSLKLADEQF	2-1	11.1
	4-1	CASSQDGLLNSYEQY	2-7	5.6
	12-5	CASGLGRGWNTEAF	1-1	5.6
	2	CASSRQLGTGELF	2-2	2.8
	4-1	CASSQDPTANSEAF	1-1	2.8
	4-1	CASSQDSTSYGYT	1-2	2.8
	4-1	CASSQEGHSNQPQH	1-5	2.8
	7-2	CASSLVLLGNSPLH	1-6	2.8
	7-9	CASSLLQGAASPLH	1-6	2.8
	19	CASSIWLSGPNYGYT	1-2	2.8
		Sequences: 36		
4-Q	27	CASGTKYYGYT	1-2	100.0
		Sequences: 50		
6-Q	27	CASSSQGYGRAQH	1-5	88.9
	3-1	CASSQYLLAGARGTYEQY	2-7	2.2
	12-4	CASSQGGVQPQH	1-5	2.2
	7-9	CASSTPSIPWSTDDFFEQY	2-7	1.1
	12-3	CASSWRVMNTEAF	1-1	1.1
	12-4	CASRPDRAGSNQPQH	1-5	1.1
	27	CASGSQGYGRAQH	1-5	1.1
	27	CASSSQGHGRAQH	1-5	1.1
	27	CASSSQGYGGAQH	1-5	1.1
		Sequences: 90		
7-I	7-9	CASSHGQGMKTQY	2-5	79.3
	20-1	CSALQLGGAGANEQF	2-1	6.9
	7-9	CANSHGQGMKTQY	2-5	3.5
	7-9	CASSLGRGMKTQY	2-5	3.5
	7-9	CASSYGQGMKTQY	2-5	3.5
	7-9	CATSPGQGMKTQY	2-5	3.5
		Sequences:29		
8-I	5-1	CASTYDRGFTGELF	2-2	96.8
	5-1	CASTYDRGFTGGLF	2-2	3.2
		Sequences: 62		
8-K	19	CASSMTYGYT	1-2	68.8
	19	CASSGSYGYT	1-2	27.1
	19	CASRMTYGYT	1-2	2.1
	19	CASSTTYGYT	1-2	2.1
		Sequences: 48		
30-K	7-6	CASSLMSGTDTQY	2-3	82.8
	18	CASSDRSQYGYT	1-2	11.8
	7-6	CASNLMSGTDTQY	2-3	1.1
	7-6	CASSLMPGTDTQY	2-3	1.1
	7-6	CASTLMSGTDTQY	2-3	1.1
	15	CATGPGAREQY	2-7	1.1
	29-1	CSVEDQTDPGYT	1-2	1.1
		Sequences: 93		
33-I	27	CASRPGQGGYEQY	2-7	73.5
	4-2	CASSQDPSGNPMETQY	2-5	14.7
	2	CASSSGTASTDTQY	2-3	2.9
	4-2	CASSPDPSGNPMETQY	2-5	2.9
	5-1	CASRYPLDLRNTGELF	2-2	2.9
	10-3	CAISGASHEQF	2-1	2.9
		Sequences: 34		
34-Q	6-6	CASTQGRGGSGYT	1-2	86.1
	3-1	CASSQAGGDAEAF	1-1	2.8
	5-1	CASSPGTANSGNTIY	1-3	2.8
	9	CASSVDAGFTDTQY	2-3	2.8
	12-4	CASSLRGDSNQPQH	1-5	2.8
	28	CASSPPSGIYNEQF	2-1	2.8
		Sequences: 36		
34-K	6-1	CASSGTSYGYT	1-2	34.9
	7-6	CASSLRRDSGPPSNQPQH	1-5	31.8
	19	CATTGQDYGYT	1-2	9.5
	27	CASSLLGTVGNQPQH	1-5	6.4
	6-1	CASSGTAYGYT	1-2	4.8
	19	CASTGGSYGYT	1-2	3.2
	20-1	CSASSGQGSGNTIY	1-3	3.2
	6-1	CASSGANYGYT	1-2	1.6
	6-1	CASSGDSYGYT	1-2	1.6
	19	CASSGQDYGYT	1-2	1.6
	30	CAWSGQVYGYT	1-2	1.6
		Sequences: 63		
20-K	7-9	CASSGTDYGYT	1-2	71.2
	7-9	CAQGGSNYGYT	1-2	5.3
	7-9	CASSSERPSNEQY	2-7	5.3
	2	CASRAQGPIYEQY	2-7	2.6
	19	CASSGTNYGYT	1-2	2.6
	19	CASTGGDYGYT	1-2	2.6
	19	CASTGPYGYT	1-2	2.6
	23-1	CASSQFDRALHQTQY	2-5	2.6
	28	CASSLSGSSYNEQF	2-1	2.6
	30	CALTRSYGYT	1-2	2.6
		Sequences: 38		
35-K	7-9	CASSPGQGFKTQY	2-5	72.6
	27	CASSPGQGGYEQY	2-7	15.7
	7-6	CASSYGQAGEQY	2-7	7.8
	7-9	CASSLGRGQETQY	2-5	2.0
	27	CASSPGQGGYGQY	2-7	2.0
		Sequences: 51		
101-K	6-1	CASTGQGYGYT	1-2	50.0
	7-9	CAEGGRDYGYT	1-2	21.4
	5-1	CASSGRNEQF	2-1	14.3
	7-9	CASSLGDRAFRNIQY	2-4	14.3
		Sequences: 14		
103-K	6-1	CASTGSYGYT	1-2	34.5
	4-3	CASSQETPRGPPLAKNIQY	2-4	20.7
	6-1	CASTGQGYGYT	1-2	13.8
	11-2	CASSISYEQY	2-7	6.9
	4-3	CASSQETPRGPPLARNIQY	2-4	3.5
	6-1	CASSGGEYGYT	1-2	3.5
	7-9	CASSPRTGPNYGYT	1-2	3.5
	19	CASNGQNYGYT	1-2	3.5
	19	CASNGRNYGYT	1-2	3.5
	19	CATTGGNYGYT	1-2	3.5
	28	CASKLAGGYYEQY	2-7	3.5
		Sequences: 29		
105-K	7-7	CASSPWGDGTDTQY	2-3	44.2
	7-9	CASSAPDSQETQY	2-5	44.2
	2	CASPREFNTEAF	1-1	4.7
	5-4	CASTTLGQDQETQY	2-5	4.7
	2	CASSDSTGVRQPQH	1-5	2.3
		Sequences: 43		
106-I	7-8	CASSFGTGGVGELF	2-2	57.5
	7-8	CASSSATGITGELF	2-2	12.5
	7-8	CASSARQGSRTDTQY	2-3	10.0
	7-8	CASSQATGNTGELF	2-2	5.0
	3-1	CASRMGANTEAF	1-1	2.5
	7-7	CASSNPDGTGDNEQF	2-1	2.5
	7-8	CASSPLTGATYNEQF	2-1	2.5
	7-8	CASSDLTGLTGELF	2-2	2.5
	7-8	CASNFGTGGVGELF	2-2	2.5
	27	CASSLSQGTILYEQY	2-7	2.5
		Sequences: 40		
107-I	7-9	CASSAGQGWKEQY	2-7	83.3
	7-8	CASSGTQVQPQH	1-5	5.6
	7-9	CASNAGQGWKEQY	2-7	5.6
	7-9	CASSAGQGWKGQY	2-7	5.6
		Sequences: 18		
107-K	7-3	CASSLIWDRGNYGYT	1-2	36.8
	25-1	CASSGSGTGYGYT	1-2	36.8
	7-6	CASSLMQGTDTQY	2-3	7.9
	19	CASSGQGYGYT	1-2	5.3
	19	CASTGTYGYT	1-2	5.3
	24-1	CATSDPNRDTDTQY	2-3	5.3
	19	CATAESYGYT	1-2	2.6
		Sequences: 38		
131-I	28	CASSRYEQY	2-7	54.2
	7-9	CASFLNTEAF	1-1	35.4
	28	CASSLYEQY	2-7	4.2
	5-5	CASSPADSQETQY	2-5	2.1
	7-9	CASFPNTEAF	1-1	2.1
	28	CASSGIGVF	1-4	2.1
		Sequences: 48		
131-K	19	CASTGSYGYT	1-2	62.2
	6-1	CASSGGSYGYT	1-2	8.9
	19	CASSGTYGYT	1-2	7.8
	19	CATTGGNYGYT	1-2	5.6
	25-1	CASSDPGQYGYT	1-2	5.6
	19	CATDTGSYGYT	1-2	4.4
	19	CASSNTYGYT	1-2	3.3
	6-7	CASSGGWYGYT	1-2	1.1
	19	CACSRSYGYT	1-2	1.1
		Sequences: 90		

[a] I, K, and Q represent HLA-B5701-restricted CD8$^+$ T-cell repertoires specific for Gag epitopes IW9, KF11, and QW9, respectively, for a given patient. Public clonotypes were defined as identical TCRβ amino acid sequences observed in more than one individual with reference to an extensive database, including both previously reported and unpublished sequences. Sequences in gray are public clonotypes that are also found in prior reports. Clonotypes shared between patients in this report are shown in color. TRBV, TCRβ variable allele; CDR3, complementarity-determining region 3; TRBJ, TCRβ joining allele.

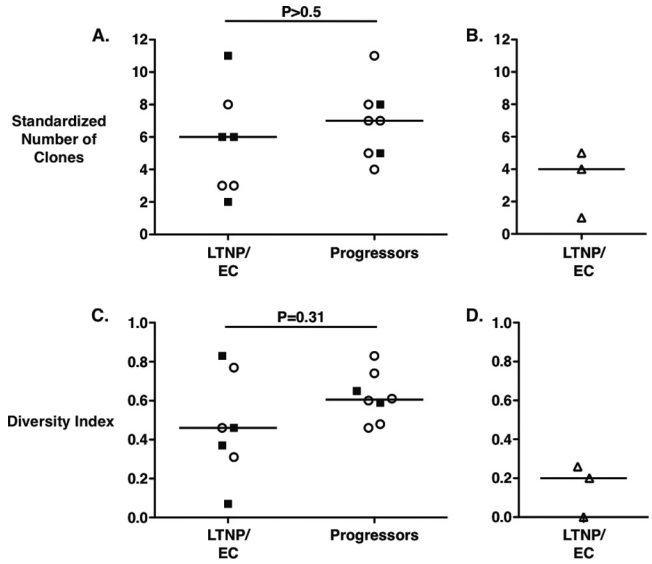

FIG 2 Comparison of HLA-B5701/HIV Gag p24 tetramer-positive CD8$^+$ T-cell repertoire diversities between HLA-B5701-positive LTNP/EC and progressors (A to D). Two diversity measures, the standardized number of clonotypes (A and B) and Simpson's diversity index (C and D), were estimated for a standard sample size of 29 TCRβ sequences per repertoire. Simpson's diversity index accounts for both the variety of amino acid sequence clonotypes and their clone sizes, ranging in value from 0 (minimal diversity) to 1 (maximal diversity). Horizontal lines indicate the median values. Squares, circles, and triangles represent the HLA-B5701-restricted HIV Gag p24-derived epitopes IW9, KF11, and QW9, respectively.

repertoire of immunodominant HIV-specific CD8$^+$ T-cell populations. We observed similar clonotype numbers and diversity in these cohorts regardless of viral load or specificity. The trend toward greater TCR polyclonality in progressors than in LTNP/EC might be explained by the recruitment of a more polyclonal response in the context of higher viral loads in the former group.

In prior work, it has been suggested that greater clonal diversity within the HIV-specific memory CD8$^+$ T-cell repertoire may mediate superior control of HIV (17, 18, 24, 38). It has been further proposed that protective HLA class I alleles might mediate their effect through thymic deletion of fewer self-reactive clones, providing a more diverse naïve repertoire (20). In either case, it is hypothesized that greater clonal diversity might mediate superior control through improved recognition of peptide variants. However, many prior studies did not include true LTNP/EC and did not compare responses within a given HLA and specificity (17, 18, 24, 38). In addition, differences in TCR contact residues within targeted epitopes, suggesting differential abilities to contain nonanchor position variation, have not been observed between B*5701-positive LTNP/EC and progressors (3, 26, 31). Therefore, if TCR clonal diversity were an important parameter differentiating LTNP/EC from progressors, some detectable difference in clonality between these groups would be expected, considering the vast differences in their capacity to control HIV replication.

It has also been suggested that LTNP/EC may possess public clonotypes that preferentially contain HIV (10, 34, 44). Public-clonotype HIV-specific CD8$^+$ T cells with high functional avidity that could be particularly efficacious might be selected in multiple individuals (15). However, we did not observe significant numbers of public clonotypes in either LTNP/EC or progressors, in contrast to prior results in which a single clonotype was found in the KF11-specific responses of 3 HLA B*5701-positive donors (10, 44). Furthermore, we previously detected no differences between LTNP/EC and progressors in the avidities of gamma interferon (IFN-γ)-secreting HIV-specific CD8$^+$ T cells responding to cognate peptides (26, 27). Selection of Gag-specific public clonotypes in the rhesus macaque-SIV infection model has been observed to correlate inversely with subsequent viral load (34). However, these studies were conducted during acute SIV infection. It is likely that the forces governing public clonotype selection in acute lentiviral infection or vaccination may differ from those involved in chronic HIV infection. It remains possible that additional study of HIV-specific CD8$^+$ T-cell clonotypes in the setting of acute infection or vaccination (42) may further our understanding of immunologic control of HIV and other chronic viral infections in humans.

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