

Crossreactive recognition of viral, self, and bacterial peptide ligands by human class I-restricted cytotoxic T lymphocyte clonotypes: Implications for molecular mimicry in autoimmune disease

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ABSTRACT The immunodominant, CD8⁺ cytotoxic T lymphocyte (CTL) response to the HLA-B8-restricted peptide, RAK-FKQLL, located in the Epstein–Barr virus immediate-early antigen, BZLF1, is characterized by a diverse T cell receptor (TCR) repertoire. Here, we show that this diversity can be partitioned on the basis of crossreactive cytotoxicity patterns involving the recognition of a self peptide—RSKFRQIV—located in a serine/threonine kinase and a bacterial peptide—RRKYKQII—located in *Staphylococcus aureus* replication initiation protein. Thus CTL clones that recognized the viral, self, and bacterial peptides expressed a highly restricted $\alpha\beta$ TCR phenotype. The CTL clones that recognized viral and self peptides were more oligoclonal, whereas clones that strictly recognized the viral peptide displayed a diverse TCR profile. Interestingly, the self and bacterial peptides equally were substantially less effective than the cognate viral peptide in sensitizing target cell lysis, and also resulted only in a weak reactivation of memory CTLs in limiting dilution assays, whereas the cognate peptide was highly immunogenic. The described crossreactions show that human antiviral, CD8⁺ CTL responses can be shaped by peptide ligands derived from autoantigens and environmental bacterial antigens, thereby providing a firm structural basis for molecular mimicry involving class I-restricted CTLs in the pathogenesis of autoimmune disease.

Epstein–Barr virus (EBV), a human gamma herpesvirus, achieves its ubiquitous distribution by a dual strategy of latency to guarantee life-long persistence and intermittent replication to ensure transmission. The probable site of viral latency is a reservoir of circulating B cells, whereas the source of infectious virus in the oropharynx is most likely reliant on an episodic supply of virus from permissively infected B cells infiltrating the mucosal epithelium (1, 2). Different forms of viral latency can be distinguished on the basis of particular patterns of virus latent gene expression (3). For instance, EBV can infect and transform B cells to grow continuously *in vitro* as lymphoblastoid cell lines (LCLs) that express the full array of the immunogenic latency proteins including EBV nuclear antigens 1–6 and latent membrane proteins 1 and 2. Transformed B cells with similar viral antigen expression represent EBV-associated polyclonal lymphomas in immunocompromised individuals (4). By contrast, it is thought that EBV persists latently *in vivo* in resting B cells that only express latent membrane protein 2A and remain immunologically silent through lack of expression of the costimulatory molecule B7 (5). The successful coexistence with its host of the most potent transforming virus known is considered to be due to immu-

nosurveillance by cytotoxic T lymphocytes (CTLs) in controlling the outgrowth of virus transformed B cells, and limiting viral replication, but without unduly compromising viral transmission (reviewed in ref. 6). A primary infection with EBV can result in the self-limiting, lymphoproliferative disease acute infectious mononucleosis. A substantial proportion of the *in vivo* activated CD8⁺ T cells recognize peptide epitopes in viral proteins associated with latency and replication (7, 8), and there is now compelling evidence that imprinting of the memory T cell repertoire can occur during primary viral or bacterial infection and remain stable over time (8–11).

Diversity within the T cell repertoire can be estimated by analyzing the distribution of T cell receptor (TCR) rearrangements. The clonally distributed $\alpha\beta$ TCR heterodimer that interacts with the major histocompatibility complex (MHC)-peptide complex presented on the surface of target cells is generated through somatic recombination of variable (V; TCRAV and TCRVB), diversity (D; TCRBD), and joining (J; TCRAJ and TCRBJ) gene segments during T cell ontogeny (12). Additional repertoire diversity is created by the imprecise joining of the different gene segments and the quasirandom insertion or deletion of nucleotides at the V-(D)-J junctional regions that span the complementarity determining region 3 (CDR3), a region of high variability in length and codon usage that makes direct contact with self and foreign peptide ligands (13, 14). The majority of CTL epitopes that have been mapped to the latent EBV nuclear antigen and latent membrane proteins, and immediate early and early lytic cycle proteins, in primary and persistent infection, are recognized by CD8⁺, class I-restricted CTLs (6), and clonal expansions in response to individual epitopes have involved the selection of highly restricted or diverse TCR repertoires (15, 16). On the other hand, a single TCR can interact with multiple peptide ligands that share, for instance, a specific motif, limited linear sequence homology or no obvious structural homology (17, 18). This aptitude for crossreactive recognition forms in essence the basis of the molecular mimicry hypothesis in which it is proposed that the sharing, in structure or sequence, of peptides between microbes and host proteins can trigger the activation of antimicrobial, antiself T cells that can initiate and/or

This paper was submitted directly (Track II) to the *Proceedings* office. Abbreviations: EBV, Epstein–Barr virus; CTL, cytotoxic T lymphocyte; LCL, lymphoblastoid cell line; TCR, T cell receptor; BZLF1, EBV immediate-early antigen; PBMC, peripheral blood mononuclear cell; RAK, RAKFQLL (residues 190–197); RSK, RSKFRQIV (residues 156–163); RRK, RRYKQII (residues 269–276); V-(D)-J, variable-diversity-joining; CDR3, compatibility determining region 3; MHC, major histocompatibility complex; PHA, phytohemagglutinin. Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. AJ235202–AJ235225).

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perpetuate autoimmune disease (19, 20). Alternatively, auto-reactivity could be induced as an outcome of bystander activation of antiseif T cells during an inflammatory response to a microbial infection (21). There is no longer a difficulty in explaining the presence of potentially autoreactive T cells in the periphery as they appear to be a normal consequence of positive selection in the thymus and are kept in control by peripheral tolerance (22–24). However, the breaking of tolerance in bystander activation could occur as an outcome of an abundant release of cytokines/autoantigens during inflammation and tissue damage, and in molecular mimicry by an increased propensity of activated antimicrobial T cells to respond to low affinity self peptide mimics (25, 26).

It is likely that EBV-infected B cells are a continual source of replicating virus throughout life-long infection (1). The pivotal switch from latency to virus replication in B cells involves the induction of the immediate-early *BZLF1* gene (27), and Epstein–Barr virus immediate-early antigen, BZLF1 (also termed ZEBRA or Zta) protein has been detected in productively infected B cells during primary infection (28). Of interest, B cells from healthy virus carriers expressed BZLF1 transcripts, but not BZLF1 protein, suggesting that cells undergoing the early stages of viral replication might be continually eliminated by BZLF1-specific CTLs. Certainly the high CTL precursor frequencies to the BZLF1-encoded epitope, RAKFKQLL (residues 190–197; referred to as RAK hereafter) (29) that are maintained in the peripheral blood of healthy, HLA-B8⁺ virus carriers could be an outcome of repeated antigen exposure (7, 30). Furthermore, the selection of diverse RAK-reactive clonotypes, in the presence of abundant antigen presentation, may indicate a low affinity TCR response that is sensitive to crossreactions with multiple peptide ligands. To investigate this possibility, we initiated a study in which RAK-reactive CTL clones were used as effectors against target cells displaying peptide homologues derived from known human pathogens or putative autoantigens. Such potentially crossreactive homologues were identified on the basis of a combination of their primary sequence similarities with the BZLF1-derived peptide, RAK, and the peptide binding motif for the restricting HLA-B8 allele (31) by searching the SwissProt database. Similar principles have been used successfully to identify peptide mimics in microbial antigens or autoantigens on the basis of structural similarity or linear sequence homology (20, 32). While there has been a general tendency to implicate MHC class II-restricted crossreactive peptides in the activation of autoreactive T cells (33), there are no *a priori* reasons for excluding a possible involvement of class I-restricted peptides in the pathogenesis of autoimmune disease. We show herein that individual MHC class I-restricted CTL clones can recognize peptide ligands, sharing linear peptide homology, from viral, bacterial, and self proteins. Moreover, multiple clones that were involved in this “three-way” molecular mimicry expressed a dominant $\alpha\beta$ TCR phenotype. These findings raise interesting issues about the possible role of CD8⁺ T cells in autoimmune disease, and the significance of self and microbial peptide ligands in shaping the peripheral T cell repertoire.

MATERIALS AND METHODS

Cell Donors and Cell Lines. Blood samples were taken from three healthy HLA-B8⁺ long-term virus carriers, SP, LC, and MM. Autologous LCLs were established from donor SP and an HLA-A1/B8 homozygous donor BM by transformation of B cells with exogenous type A EBV (QIMR-WIL isolate). LCLs were routinely maintained in growth medium consisting of RPMI 1640 medium, 2 mM glutamine, 100 units/ml penicillin, 100 μ g/ml streptomycin, and 10% (vol/vol) heat-inactivated fetal bovine serum. Phytohemagglutinin (PHA) blasts were generated from donors SP, LC, MM, and BM as described (34).

Selection and Synthesis of Peptides. Peptide homologues were identified on the basis of a combination of their primary sequence similarities with the BZLF1-derived peptide, RAK, and the peptide binding motif for HLA-B8 by searching the SwissProt database using the motif, X-X-[KR]-[FY]-[KR]-[QN]-[LIVM]-[LIVM]; amino acid sequence is denoted in standard single letter code, where X represents any amino acid. Seven peptide homologues used in the present study were selected from a vast pool of identified sequences on the basis of whether they were derived from known human pathogens or putative autoantigens. All selected peptides were purchased from Chiron Technologies (Melbourne, Australia).

Agar Cloning of T Cells. Peripheral blood mononuclear cells (PBMCs) from donor SP were activated by stimulation with the γ -irradiated (80 Gy) autologous LCL or an LCL from the HLA-A1/B8 homozygous individual BM at a stimulator/responder cell ratio of 1:20, and T cell clones were generated as described (35). Clones were maintained in growth medium containing recombinant interleukin 2 (Chiron Technologies) and were routinely immunophenotyped for CD3, CD4, and CD8 markers as described (34).

Cytotoxicity Assay. CD8⁺ RAK-reactive CTL clones from donor SP were used as effectors against PHA blasts pulsed with the appropriate peptide (100 μ g/ml, 0.2 ml). Target cells, with or without peptide, were incubated with 100 μ Ci (1 Ci = 37 GBq) of ⁵¹Cr at 37°C for 90 min, washed twice by centrifugation, and used in standard 4-h ⁵¹Cr-release assays.

Limiting Dilution Analysis. The memory CTL precursor frequencies to the peptides, RAK, RSKFRQIV (residues 156–163; referred to as RSK hereafter), and RRKYKQII (residues 269–276; referred to as RRK hereafter), were quantified by using limiting dilution analysis as described (34).

Amplification and Sequencing of Rearranged TCR α and β Sequences. TCR α rearranged sequences were amplified with one of 32 5' TCRAV family-specific oligonucleotides (V α 1–32) and a 3' TCRAC(C α) constant primer. Amplification conditions were the same as those described (16) except that annealing was performed at 55°C. The oligonucleotides V α 1–12/V α 17–18/C α and V α 13–16/V α 22–29/V α 32 were synthesized according to Davies *et al.* (36) and Steinle *et al.* (37), respectively. The V α 15, 16, and 17 primers reported previously (36) were reassigned to V α 19, 20, and 21, respectively, in accordance with the new nomenclature (38). TCR α families 30 and 31 were amplified with the following oligonucleotides: V α 30, 5'-CTTACCCTGTATTCAGCTGGG-3'; V α 31, 5'-CTGCAGCTTCTTCAGAGAGACAATGG-3'. TCR β rearranged sequences were amplified as detailed (16). TCRBV25 was amplified under the conditions described (16) with the following oligonucleotide: VB25, 5'-AACAGGTCCTGAAAACGAGTTCAA-3'. The amplified TCR β and TCR α sequences were purified and sequenced as reported (16).

RESULTS AND DISCUSSION

CD8⁺ (>99% purity), RAK-reactive CTL clones were generated from an HLA-B8⁺ healthy virus carrier, SP. Remarkably, clones were identified that also crossreacted with two of the peptide homologues, RSK from a human serine/threonine kinase (GenBank accession P27448) and RRK from *Staphylococcus aureus* replication initiation protein (GenBank accession P14490). The ⁵¹Cr-release data in Fig. 1 show the cytotoxicity profiles of three clones responding to HLA-B8⁺ PHA-blasts pulsed with selected peptide homologues. Three crossreactivity patterns were distinguished: (i) clone SP42 strongly lysed targets pulsed with RAK, RSK, or RRK peptides (referred to as triple peptide-reactive clones hereafter), (ii) clone SP35 recognized both the RAK and RSK peptides (referred to as double peptide-reactive clones hereafter), and (iii) clone SP28 stringently lysed targets pulsed with the RAK peptide (referred to as single peptide-reactive clones hereafter).

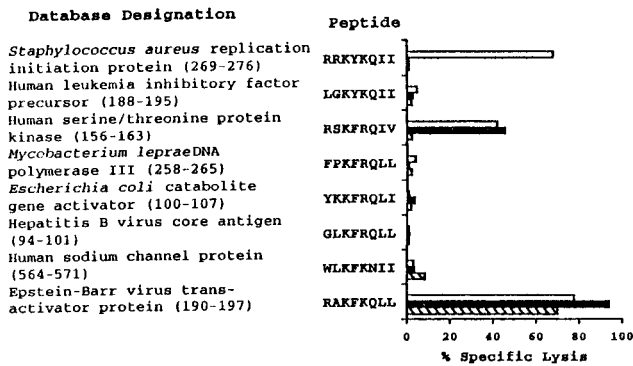


FIG. 1. Specific lysis shown by three selected CTL clones generated from an HLA-B8⁺, healthy virus carrier SP after stimulation *in vitro* of PBMCs with the autologous LCL. Clones were tested in standard 4-h ⁵¹Cr-release assays against autologous PHA blasts pulsed with the BZLF1-associated RAK peptide or peptide homologues derived from known human pathogens or putative autoantigens, as listed on the ordinate. Amino acid positions in the protein sequence are given in parentheses. Triple peptide-reactive clone SP42 (□), double peptide-reactive clone SP35 (■), and single peptide-reactive clone SP28 (▨). Effector/target ratio, 2:1.

The discovery of a potential self peptide that might be involved in positive selection of a subpopulation of RAK-reactive CTLs prompted a more detailed study of these crossreactions in donor SP. An analysis of 220 CD8⁺, RAK-reactive CTL clones provided estimates of the abundance of clones belonging to each of the crossreactivity groups. A predominant number of clones (52.7%) were single peptide-reactive, 36.8% of the clones were double peptide-reactive, and 10.5% were triple peptide-reactive. The fine specificities of 10 representative clones from each group are shown in Fig. 2 *a-c*. The EBV nuclear antigen 3-encoded, HLA-B8-restricted peptides, FLRGRAYGL and QAKWRLQTL, included in each ⁵¹Cr-release assay as specificity controls, were not recognized by the RAK-reactive clones. The abundance and specificity of the crossreactive clones made it highly unlikely that they were "forbidden" clones or expressed degenerate TCR recognition. Instead, it is more likely that they represent a subpopulation of potentially autoreactive T cells normally found in the periphery as an outcome of intrathymic positive selection (23, 24, 39). There is good evidence that positive selection of the mature T cell peripheral repertoire is based on low-avidity interactions between the $\alpha\beta$ TCR on thymocytes and self peptide ligands presented by MHC molecules expressed on thymic stromal cells (24). In the periphery, these autoreactive T cells would only be weakly-reactive to self peptides because the avidity required for TCR activation during positive selection of thymocytes evidently is lower than that required for TCR activation during the recognition of antigen by mature T cells. These constraints on autoreactivity can obviously be circumvented by activating CTLs *in vitro* with the cognate peptide or *in vivo* after an appropriate infection.

To gain insight into the affinity properties of the RAK, RSK, and RRK peptides, we characterized their ability to sensitize target cell lysis by testing representative clones selected from each of the crossreactivity groups for their response to varying concentrations of peptides. The peptide titration results in Fig. 3 *a-c* show that the RAK peptide was substantially more effective than the RSK and RRK peptides in sensitizing targets to lysis, and that all clonotypes were virtually equally effective in the lysis of RAK-sensitized targets. However, the RSK and RRK peptide concentrations required for optimal target sensitization was 20- to 30-fold higher than for the RAK peptide. Another difference in peptide recognition was reflected at the level of inhibition of lysis with anti-class I mAb as lysis of targets pulsed with RSK or RRK peptide was considerably

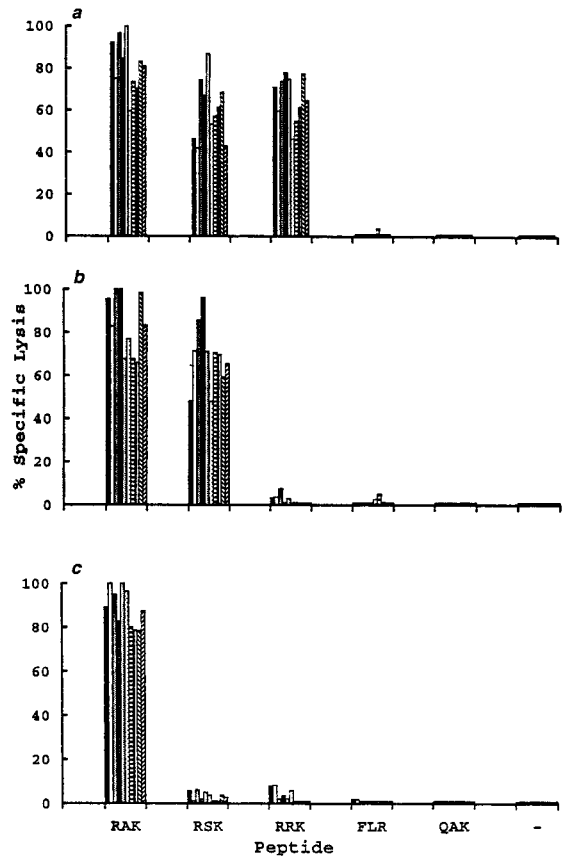


FIG. 2. Specific lysis shown by representative triple (*a*), double (*b*), and single (*c*) peptide-reactive CTL clones generated from an HLA-B8⁺, healthy virus carrier SP after stimulation *in vitro* of PBMCs with the autologous LCL or HLA-A1/B8 homozygous BM LCL. Clones were tested in standard 4-h ⁵¹Cr-release assays against autologous or BM PHA blasts pulsed with RAK, RSK, RRK, FLR, or QAK peptide, or without added peptide (-). Effector/target ratio, 2:1.

more sensitive to inhibition than targets pulsed with the RAK peptide (I.S.M., unpublished data). The simplest explanation for these differences is that both the self and bacterial peptides behave as low-affinity ligands for the TCR expressed by the RAK-reactive CTLs.

The weaker cytotoxic responses obtained with limiting concentrations of RSK and RRK peptides indicated that these homologues also might behave differently to the cognate peptide at the level of activation of memory CTLs, as has been observed with altered peptide ligands (reviewed in ref. 40). Accordingly, the precursor frequencies of memory CTLs responding to the RAK, RSK, and RRK peptides were estimated by limiting dilution analysis of PBMCs from three HLA-B8⁺, healthy virus carriers. The limiting dilution analysis outcomes for donors SP, LC, and MM are shown in Fig. 4 *a, b, and c*, respectively. Clearly, all RAK peptide-stimulated cultures showed high precursor frequencies of CTLs that recognized RAK, RSK, or RRK peptide, and, invariably, the highest frequencies were associated with recognition of the RAK peptide and the lowest with the RRK peptide. This hierarchy confirmed the clonal CTL findings in donor SP. An analysis of individual microcultures at the lower responder cell levels also provided evidence of triple peptide-, double peptide-, and single peptide-reactive CTL precursors (data not shown). By contrast, considerably weaker frequencies were detected in the RSK peptide- and RRK peptide-stimulated cultures, indicating that these peptides were poorly immunogenic in activating memory T cells. We verified that the RSK peptide sequence is present in the genomic DNA derived from

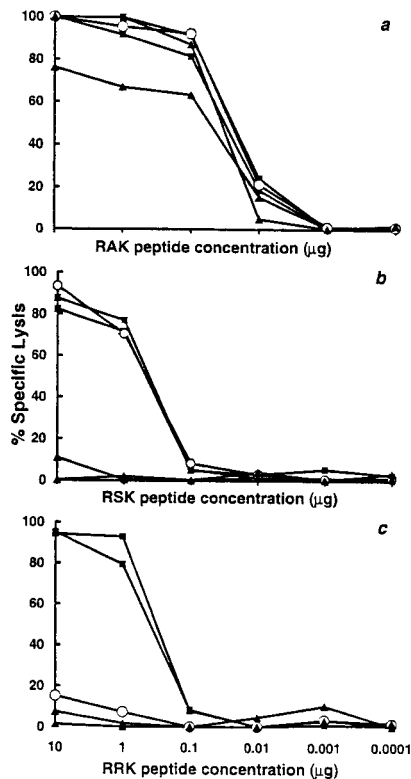


FIG. 3. Specific lysis shown by representative triple (■), double (○), and single (▲) peptide-reactive CTL clones generated from an HLA-B8⁺ healthy virus carrier SP after stimulation *in vitro* of PBMCs with the autologous LCL or HLA-A1/B8 homozygous BM LCL. Clones were tested in standard 4-h ⁵¹Cr-release assays against autologous PHA blasts pulsed with varying concentrations of RAK (a), RSK (b), or RRK (c) peptide. Effector/target ratio, 2:1.

the autologous LCL of donor SP (I.S.M. and S.C., unpublished data), although it is not known whether this self peptide is processed or presented naturally. Nevertheless, in theory, the RSK peptide could behave as a low-affinity ligand resulting only in the weak reactivation of specific memory T cells due to peripheral tolerance. Ligation of the TCR by altered peptide ligands can result in diminished signaling events involving the incomplete tyrosine phosphorylation of TCR subunits and no activation of ZAP-70 (41). Such modified TCR signaling, particularly in response to tolerizing peptides, could instigate T cell anergy, ignorance, or deletion, rather than activation. Intriguingly, the bacterial peptide behaved like a tolerogenic self peptide. Because *S. aureus* commonly forms part of the mucosal commensal flora (42), it is perhaps not unreasonable to expect that host T cells may be tolerant to protein antigens derived from this commensal, akin to the systemic tolerance that can be induced in T cells by antigens from other commensals such as enteric bacteria, and by dietary antigens (43, 44).

Next, we defined the $\alpha\beta$ TCR phenotype of selected RAK-reactive CTL clones from donor SP to determine whether there was a structural basis to accommodate the different crossreactivity patterns observed with multiple peptide ligands. Analysis of the TCR $\alpha\beta$ sequences, identified by using TCRAV and TCRBV family-specific PCR followed by direct sequencing, showed several interesting features as presented in Fig. 5 *a* and *b*. Comparison of the predicted amino acid sequences of all triple peptide-reactive TCRs revealed identical TCRAV3AJ16S5 rearrangements with an identical complementarity determining region 3 (CDR3) that directly contacts the peptide epitope. This conserved V α chain was paired predominantly to TCRBV7BJ2S7; clone SP4 was paired

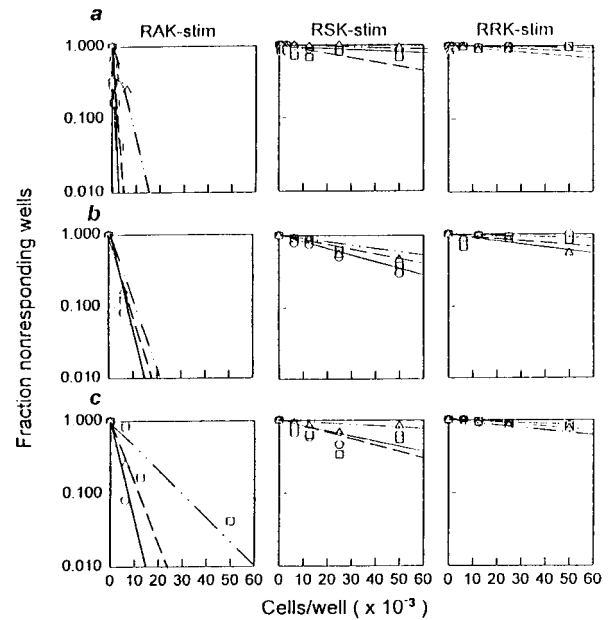


FIG. 4. Comparative CTL precursor (CTL_p) frequencies in PBMCs from three HLA-B8⁺ healthy virus carriers SP (a), LC (b), and MM (c) by using limiting dilution analysis. PBMCs were stimulated with autologous PHA blasts pulsed with RAK, RSK, or RRK peptide. The frequency of CTL_ps responding to individual peptides was estimated from split-well assays by using standard 4-h ⁵¹Cr-release assays and autologous or BM PHA blast targets pulsed with RAK (○), RSK (△), or RRK (□) peptide.

to TCRBV6BJ2S7 and used a distinct V β chain CDR3 sequence, although there was a constrained maintenance of CDR3 loop length, indicating antigen-driven selection. The detection of triple peptide-reactive CTL clones with identical TCRs is not simply due to the amplification *in vitro* of sister clones because the precursor T cells giving rise to these clones were initially stimulated in separate cultures before agar cloning. Also, the recurrent $\alpha\beta$ TCR expressed by the triple peptide-reactive clones was found in donor SP on three separate occasions. By comparison, the double peptide-reactive TCRs showed sequence variation, albeit limited, in both the V α and V β chains. Nonetheless, clones expressing identical TCRs were identified. Of interest, clone SP1 expressed a V β chain identical with that of the conserved triple peptide-reactive TCR, but was paired to a different V α chain, confirming the triple peptide-reactive TCR findings that the V α chain is important in the specific recognition of the bacterial peptide. Analysis of single peptide-reactive clones revealed no identical V α /V β rearrangements, and each clonotype expressed a distinct CDR3 amino acid composition of variable length. This outcome resembled the broad diversity obtained in an earlier TCR V β analysis of RAK-reactive clonotypes (16). Constraints on the structural plasticity of the triple peptide-reactive TCRs were also obvious at the level of epitope recognition as determined by using peptide analogues in which each residue of RAKFKQLQ was sequentially substituted by alanine. Not surprisingly, all triple peptide-reactive clones interacted uniformly and uniquely with the alanine replacement set in that substitutions were not tolerated at positions 3, 4, 6, and 7 while the fine specificity patterns of the double peptide- and, particularly, the single peptide-reactive clones showed multiple profiles, although all RAK-reactive clones were equally sensitive to substitution at position 4, which may represent a critical TCR contact site (I.S.M., unpublished data). Overall, the TCR data clearly demonstrate that a diverse RAK-reactive TCR repertoire can be partitioned and shaped on the basis of focused crossreactions with

a		CTL	TCRAV	FW	CDR3	FW	TCRAJ	CDR3 Length
RAK/RSK/RRK								
SP3, 4, 18, 24, 27, 42	AV3	C A T	W M D S S Y K L	ggt gct acg	tcg acg gat agc agc tac aaa ttg	I F G	AJ1655	8
RAK/RSK/*								
SP1, 12, 14, 16, 23, 35	AV10	C A G	A D R D D K I	tgt gca gga	gcg gac aga gat gac aag atc	I F G	AJ954	7
SP17	AV17	C A A	S G M G Q N P	C A A	S G M G Q N P	V P P	AJ1352	7
SP25	AV19	C A V	A P P G G A D G L	tgt gca gca	agc gga aat ggt cag aat ttt	T F G	AJ957	9
RAK/*/*								
SP2	AV15	C A B	S E T G A N N L	tgt gca gag	agt gaa act ggg gca aac aac ctc	P P G	AJ1755	8
SP6	AV20	C L V	G S I T D D M R	tgt gct gtc	aag agc ccc tct tct aga cgg cca gaa ctg	P G A	AJ157	8
SP8	AV2	C A V	S T S G G A D G L	tgt gct gtc	agt acg gaa gga gga gtc gct gac gga ctc	I F G	AJ957	10
SP20	AV15	C A B	M S T S Y D X V	tgt gca gag	atg gaa acc tcc tac gac aag gtg	I F G	AJ1353	8
SP43	AV7	C A V	F S G S A R Q L	tgt gca gag	atg gaa acc tcc tac gac aag gtg	T F G	AJ1653	8
	AV27	C A V	T S G T Y K Y	tgt gct gct	ttt tct ggt tct gca agg caa ctg	I F G	AJ17510	8

b		CTL	TCRBV	FW	CDR3	FW	TCRBJ	CDR3 Length
RAK/RSK/RRK								
SP10, 18, 24, 26, 27, 42	BV7	C A S	S Q K G Q L S Y E Q	tgc gcc agc agc caa aaa gga cag ctg tcc tac gag cag	tcg tcc tac gag cag	Y F G	BJ257	10
SP4	BV6	C A S	S P P G Q G I N Y E Q	tgc gcc agc agc ccc ccg gga cag ggg att aac tac gag cag	tcg tcc tac gag cag	Y F G	BJ257	11
RAK/RSK/*								
SP12, 16, 23, 35	BV7	C A S	S H Q I G L A G G B Q	tgt gcc agc agc cat caa atc gga cta gca gga ggg gag cag	tcg tcc tac gag cag	F F G	BJ251	11
SP1	BV7	C A S	S Q K G Q L S Y E Q	tgc gcc agc agc caa aaa gga cag ctg tcc tac gag cag	tcg tcc tac gag cag	Y F G	BJ257	10
SP7	BV3	C A S	S L A F R S I G N T G B L	tgt gcc agc agc ttc gca ttt agt tct att ggg aac acc ggg gag ctg	tcg tcc tac gag cag	F F G	BJ252	13
SP17	BV7	C A S	S Q G L V T S G R D N H Q	tgt gcc agc agc caa ggc cta gta act agc ggt cgg gac aat gag cag	tcg tcc tac gag cag	F F G	BJ251	13
SP25	BV4	C S V	N T G E W D B Q	tgt gcc agc agc caa ggc cta gta act agc ggt cgg gac aat gag cag	tcg tcc tac gag cag	Y F G	BJ257	8
RAK/*/*								
SP2	BV12	C A I	S D H K R T G G T E A	tgt gcc atc agc gac cat aag agg aca ggg ggc act gaa gct	tcg tcc tac gag cag	F P G	BJ151	11
SP8	BV6	C A S	S ? L E S D R G T G N T I	tgt gcc agc agc tta gtt ctc cta cgg ggc ccc aca gat acg cag	tcg tcc tac gag cag	F F G	BJ253	12
SP20	BV6	C A S	S S T A P G L A G F R Q	tgt gcc agc agc tca act gct ccg gga cta ggc gga ttc cgg cag	tcg tcc tac gag cag	Y F G	BJ257	12
SP43	BV14	C A S	S ? L E S D R G T G N T I	tgt gcc agc agc ccc cta gaa tcc gac agy ggy act gga aac acc ata	tcg tcc tac gag cag	T F G	BJ153	13

Fig. 5. V-(D)-J junctional region sequences of α (a) and β (b) chains expressed by triple-peptide (RAK/RSK/RRK), double-peptide (RAK/RSK/*), or single-peptide (RAK/*/*) reactive CTL clones generated from an HLA-B8⁺ healthy virus carrier SP. A translated amino acid sequence is shown above each corresponding nucleotide sequence that extended at least 70 nucleotides further 5' of the sequence shown. CTL clones are listed on the ordinate and those expressing identical TCR sequences are grouped together. For each clone, the deduced amino acid sequence of the CDR3 loop is shown putatively supported by two framework branches (FW) and the CDR3 amino acid length is reported. For certain clones only the α chain or β chain sequence was determined.

peptide homologues derived from an autoantigen and an environmental bacterial antigen. However, by extending the limited panel of homologues used in this study, we anticipate that additional crossreactive peptides will be discovered, some of which may be responsible for further partitioning of the diverse RAK-reactive repertoire.

Interestingly, the finding of recurrent TCRs, combined with the three distinct patterns of killing shown by the RAK-reactive CTLs, suggests that clonal expansion of the triple peptide-reactive CTLs is antigen driven and not simply an outcome of fortuitous crossreactions generated in culture. To explain this finding, it is plausible to assume that T cells are normally tolerant to the self and bacterial peptides due to peripheral and mucosal tolerance, respectively. Because low-avidity interactions between TCR and self peptide/MHC molecules have been implicated recently in the long-term survival *in vivo* of naive and memory T cells in the absence of the cognate antigen (45, 46), the bacterial peptide could perform a similar role in contributing to the maintenance and survival of a pool of triple peptide-reactive CTL precursors. Precisely how the self and bacterial peptides "cross-prime" RAK-reactive CTLs is conjectural, although relevant clues may be found in the common topography of intermittent *S. aureus* and persistent EBV co-infections in mucosal regions such as the nasopharynx and tonsillar lymphoepithelium. Importantly, tolerance to the self peptide could be disrupted periodically by molecular mimicry or bystander activation at sites of inflammation during an immune response to recrudescence EBV, and to the bacterial peptide during a skin or invasive infection with *S. aureus*. Available abundant bacterial and self antigens ostensibly could then be processed by den-

dritic cells through an exogenous vacuolar class I pathway for presentation to CD8⁺ T cells (47, 48), either locally in the peripheral tissue if the T cells have been preactivated by the cognate peptide or in the regional lymph nodes if they are naive or resting memory cells, thereby resulting in their clonal expansion. Such clonal expansions could presumably take place before a primary infection with EBV resulting in a high frequency of naive RAK-reactive CTL precursors poised to recognize the cognate peptide. Indeed, we have found that a dominant polyclonal response of *in vivo*-activated RAK-reactive CTLs derived from an HLA-B8⁺ acute infectious mononucleosis donor included clones that strongly crossreacted with the RSK and RRK peptides (I.S.M., unpublished data). Moreover, it will be interesting to determine whether the exceptionally dominant, polyclonal RAK-reactive responses that have been identified in acute infectious mononucleosis donors by using tetramer technology (7) also consist of clones crossreactive for multiple peptide ligands. Future studies with tetramers should clarify the importance of crossreactions in imprinting and selection of the peripheral TCR repertoire responsive to the immunodominant RAK peptide.

Potentially self-reactive T cells in the periphery are obviously kept under rigorous control by self-tolerance and ordinarily would not be expected to pose an autoimmune threat. Thus in the context of this study, the viral peptide, but not the self peptide, was effective in the reactivation *in vitro* of RAK-reactive memory T cells. However, once activated by the cognate peptide, crossreactive CTLs, if present in sufficient numbers and in conditions of abundant self/bacterial antigen presentation, could conceivably contribute by cytokine release or cytolytic activity to the immunopathology at sites of sus-

tained inflammation. Indeed, it has been suggested that the BZLF1-specific CTLs found in considerable numbers in the afflicted joints of patients with chronic rheumatoid arthritis exacerbate disease (49). Although there is evidence of EBV infection in synovial tissue (50), implying that the antiviral CTLs could be responding to local antigenic stimulation, the detection of latent or lytic EBV infection can be infrequent (51). However, the absence of EBV could simply indicate that an earlier infection had been cleared by CTL intervention. This outcome could be expected if there was a dominant presence of EBV-specific CTLs in the synovium, unless these effectors were in some way immunocompromised *in situ*. There is also the possibility that EBV infection in the synovial membrane is episodic and dependent on a supply of virus from EBV-infected B cells within the synovium. We can now predict, on the basis of our findings, that antiviral bystander effects could also be potentiated by crossreactions involving self or bacterial peptides resulting from molecular mimicry, bystander activation and/or epitope spreading (52) occurring during the disease process. In fact, a possible scenario is that MHC class I-restricted crossreactive CTLs are perpetuated at inflammatory sites in response to elevated levels of self or bacterial peptides presented by local dendritic cells without a need for an ongoing EBV infection to provide continual cognitive stimulation. Of course, other viruses apart from EBV could be associated with the pathogenesis of chronic rheumatoid arthritis through crossreactions, in a fashion similar to the implied events occurring in autoimmune diseases such as multiple sclerosis, in which patients can exhibit exacerbations after infections with different viruses (53).

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