Different HLA-B27 Subtypes Present the Same Immunodominant Epstein-Barr Virus Peptide

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Summary

An immunological basis has been postulated for the strong association between at least five subtypes of the HLA-B27 allele (B27.01, .02, .04, .05, and .06) and ankylosing spondylitis, namely that cytotoxic T lymphocyte (CTL) responses are induced against an "arthritogenic" peptide that these different subtypes can all present. This requires a degree of overlap between the peptide binding repertoires of different B27 molecules. The present work, using CTL responses to Epstein-Barr virus (EBV) as a model system in which to identify B27-restricted epitopes, provides the first direct evidence that different disease-related alleles can present the same immunodominant peptide. We first noted that EBV-specific CTL clones, whether from B27.05-, B27.02-, or B27.04-positive donors, were largely subtype-specific in their restriction, recognizing only EBV-transformed B cell lines of the relevant B27 subtype. However, when tested against targets expressing individual EBV proteins from recombinant vaccinia virus vectors, all B27.05-restricted, all B27.02-restricted, and a proportion of B27.04-restricted clones were reactive to the same viral nuclear antigen, Epstein-Barr nuclear antigen (EBNA)3C. In subsequent peptide sensitization assays, all the EBNA3C-specific clones tested and also the EBNA3C-specific component within polyclonal CTL preparations from B27.05-, B27.02-, or B27.04-positive donors recognized the same immunodominant viral peptide RRIYDLIEL (EBNA3C residues 258–266). This sequence accords well with the proposed B27.05 peptide motif and clearly must be accommodated within the different peptide binding grooves of B27.05, B27.02, and B27.04 molecules. Clonal analysis revealed a second component of the B27.04-restricted response that was not shared with other subtypes. This was directed against an EBV latent membrane protein LMP2 epitope whose sequence RRRWRRLTV satisfies some but not all requirements of the B27.05 peptide motif. We conclude that there is indeed a degree of functional overlap between different B27 subtypes in their selection and presentation of CTL epitopes.

The role of HLA class I molecules is to bind peptide fragments of endogenously synthesized cellular or viral proteins and to present these as HLA–peptide complexes at the cell surface for recognition by CTLs (1–4). Stability of the complex requires a high affinity interaction between the peptide and the peptide binding groove (5). Because the precise structure of the groove is affected by HLA polymorphism (6), any one particular class I allele will present a particular selection of peptides (7). The concept is best illustrated with reference to the HLA-B27.05 molecule, for which a detailed x-ray crystallographic structure is now available (8, 9) and where elution and microsequence analysis of naturally processed peptides has identified certain shared features of a B27.05 allele-specific peptide motif (10). In this motif, the consistent presence of an arginine at position 2 can be understood in terms of the high affinity of this amino acid's side chain for the "B" pocket in the B27.05 peptide binding groove. Likewise, other structural constraints appear to favor the presence of hydrophobic residues at position 3 and of positively charged amino acids at the NH_2- and COOH-terminal positions 1 and 9 (8–10).

HLA-B27.05 is the most common of seven distinct subtype alleles (B27.01–B27.07) which together make up the serologically defined HLA-B27 family of class I molecules. These subtypes differ from one another in a limited number of amino acid residues occupying defined positions in the α-helical sides or β-pleated sheet floor of the peptide binding groove (11, 12). Interest in the B27 family stems from its very strong linkage with ankylosing spondylitis and related spondyloarthropathies (13, 14). This represents by far the clearest example of an HLA-linked disease in humans, and, indeed, there are strong grounds for believing that HLA-

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B27 molecules are directly involved in the disease process (15), probably as targets of an immunopathological response. Of several mechanisms proposed in this regard (16), one of the most interesting postulates a role for CTL responses directed against an "arthritogenic" peptide, of cellular or microbial origin, which is selectively presented by HLA-B27 (17). In this context, at least five of the above B27 subtypes are known to be disease linked. These are the widespread B27.05 subtype, the B27.01 and B27.02 subtypes found in Caucasian populations, and the B27.04 and B27.06 subtypes found in South East Asian populations (16, 18). The implication is, therefore, that all five B27 subtype molecules must be capable of binding and presenting the same arthritogenic peptide despite differences in the structure of their peptide binding grooves.

Leaving aside the specific question of the identity of such an arthritogenic peptide, here we address the more general question that this interpretation raises, namely, is there demonstrable overlap between the range of peptide antigens presented as CTL epitopes by different B27 molecules? The model system we have used for this purpose involves CTL responses to EBV, a herpesvirus widespread in human populations and carried by all previously infected individuals as a latent infection of B lymphoid tissues (19). All healthy virus carriers possess memory CTLs to EBV, and these can be reactivated by appropriate in vitro stimulation with the autologous EBV-transformed B lymphoblastoid cell line (LCL)[1] (20). The present work identifies the dominant viral target epitopes presented by three different B27 subtypes, B27.05, B27.02, and B27.04, each of which is disease linked. Remarkably, despite the number of viral proteins available for recognition in this herpesvirus system, all three B27 subtypes present the same immunodominant viral peptide.

Materials and Methods

B27-positive Donors. Six B27-positive individuals, all previously infected by EBV, were used in this work: SC (HLA-A2, A2, B27.05, B27.05), RT (HLA-A2, A24, B27.05, B35), EN (HLA-A1, A2, B18, B27.05), LY (HLA-A1, A24, B27.02, B35), DH (HLA-A2, A11, B27.04, B40), and DW (HLA-A2, A24, B13, B27.04).

Target Cells. EBV (B95.8 strain)-transformed LCLs were established from each of the above donors by in vitro infection of PBMC; other LCLs from donors of known B27 subtype were kindly provided by Professor A. J. McMichael (University of Oxford, Oxford, UK), Dr. L. MacLean (Northwick Park Hospital, London, UK), Dr. B. S. Breur-Vriesendorp (Central Laboratory of Netherlands Red Cross, Amsterdam, The Netherlands), and Dr. A. Lopez de Castro (University of Madrid, Madrid, Spain). EBV-negative target cells included PHA-stimulated T lymphoblasts maintained in IL-2-conditioned medium and used in CTL assays from 14 d after stimulation, and fibroblasts established from skin biopsies and maintained in standard monolayer culture.

Generation of EBV-specific CTL. PBMC from the above donors were cocultivated with γ-irradiated cells of the autologous EBV (B95.8)-transformed LCL (responder to stimulator ratio of 40:1), and the resultant EBV-specific polyclonal CTL preparations maintained in IL-2–conditioned medium as described (21). EBV-specific CTL clones were generated from cocultures at day 4 after stimulation by seeding in semi-solid agarose as described (22). After establishment, clones were maintained by weekly refeeding in medium containing 20 U/ml rIL-2 (kindly provided by Glaxo Ltd., London, UK) and 20% vol/vol supernatant of the IL-2–producing MLA-144 cell line, and by weekly stimulation with γ-irradiated autologous LCL cells.

Vaccinia Virus Recombinants. The generation of vaccinia virus recombinants encoding the EBV latent proteins (Epstein-Barr nuclear antigens [EBNAs] 1, 2, 3A, 3B, 3C, LP, and latent membrane proteins [LMP]1 and 2) has been described previously (23, 24). All coding sequences for EBV latent proteins were of B95.8 strain origin (25).

Synthetic Peptides. Peptides were synthesized using fluorenylmethoxycarbonyl chemistry by Dr. J. Fox (Alta Bioscience, University of Birmingham). Peptides were dissolved in DMSO and assayed for protein concentration by a modification of the Biuret assay (26) before storage at −20°C.

Cytotoxicity Assays. Effector CTLs from each donor were screened in a standard 4-h ^{51}Cr-release assay. E/T ratios were between 2:1 and 8:1 for CTL clones and between 5:1 and 15:1 for polyclonal CTL preparations. The use of recombinant vaccinia virus–infected fibroblasts in cytotoxicity assays has been described previously (24). Briefly, monolayer cultures of fibroblasts were exposed to recombinant vaccinia virus (multiplicity of infection 10) for 2 h, then additional culture medium added for 16 h. Infected cultures were then harvested by trypsinization, labeled for 1 h with $^{51}CrO_4$, washed three times, and used as targets in the standard CTL assay.

In peptide sensitization assays, PHA blasts were labeled with $^{51}CrO_4$ for 2 h, washed twice, then incubated with peptide at a concentration of either 40 μg/ml for 15-mer peptides or 4 μg/ml for 10/9-mer peptides in a volume of 100 μl. After 1 h, CTLs were added in a 100-μl volume to give final peptide concentrations of 20 and 2 μg/ml, respectively for the duration of the assay.

Results

B27 Subtype Restriction of EBV-specific CTL Responses. An initial series of experiments asked to what extent EBV-specific CTLs reactivated in vitro from donors of known B27 subtype were capable of recognizing EBV-positive LCL targets of other subtypes. Since the B27-positive donors to be studied shared certain other HLA class I alleles, it was necessary to analyze their B27-restricted effectors as clones rather than within polyclonal CTL populations. Clones showing operational specificity for EBV (i.e., recognizing the autologous LCL but not autologous PHA blasts) and B27 restriction (i.e., recognizing a B27 subtype-matched LCL) in initial screening assays were expanded and tested against a panel of LCL targets expressing one of six different B27 subtypes, B27.01-B27.06.

Fig. 1 shows data from representative clones derived from a B27.05-positive donor SC, a B27.02-positive donor LY, and a B27.04-positive donor DH. All the B27.05-positive SC clones (SCc2 and SCc39 are representative of 10 clones tested) were entirely subtype-specific in their restriction, showing clear recognition of B27.05 LCL targets but not of any other LCLs in the B27 panel. Similar results were obtained with five CTL clones analyzed from a second B27.05-positive donor RT (data not shown). Of the B27.02-positive LY clones tested 7/33 (e.g., LYc21) were also subtype specific, recognizing only B27.02 LCL targets. However, another 26 LY clones (e.g.,

[1] *Abbreviations used in this paper:* EBNA, Epstein-Barr nuclear antigen; LCL, lymphoblastoid cell line; LMP, latent membrane protein.

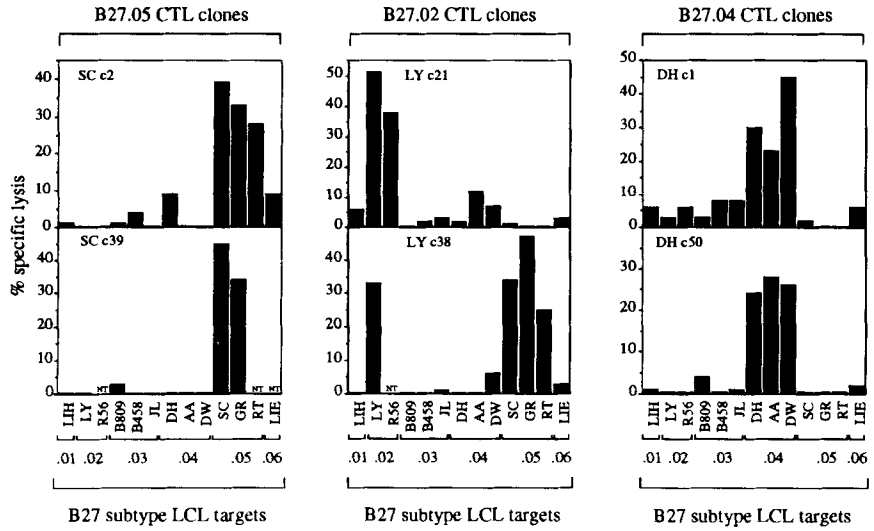

Figure 1. B27 subtype restriction of EBV-specific CTL responses. Representative B27-restricted CTL clones from B27.05-positive donor SC (A2, B27.05), B27.02-positive donor LY (A1, A24; B27.02, B35), and B27.04-positive donor DH (A2, A11; B27.04, B40) were tested against a panel of LCL targets (including the autologous LCL) representing the six B27 subtypes B27.01–B27.06. E/T ratios were between 2:1 and 8:1. Results are expressed as percent specific lysis and are illustrative of those in several repeated assays.

LYc38) were capable of recognizing not only B27.02, but also B27.05 LCLs. All B27.04-positive DH clones assayed on the same panel (DHc1 and DHc50 are representative of 23 clones tested) were specific for B27.04 LCL targets only.

EBV Target Antigen Specificity of B27-restricted CTL Responses. Viral antigen expression in EBV-transformed LCLs is thought to be limited to eight latent proteins. These are EBNAs 1, 2, 3A, 3B, 3C, and -LP, and LMPs 1 and 2 (27). In a recent study, we mapped the viral antigens being recognized by polyclonal EBV-specific CTL populations by using recombinant vaccinia virus vectors to express these latent proteins individually in appropriate target cells (24). Adopting the same approach here, as many as possible of the above B27-restricted CTL clones were then assayed on autologous fibroblast targets infected with the recombinant vaccinias.

The results are illustrated in Fig. 2, again using data from the same representative clones. All 8 B27.05-positive SC clones tested in this way (e.g., SCc2, SCc39) recognized targets expressing the EBNA3C viral protein, whereas lysis of all other vaccinia-infected targets remained at background levels. A similar result was obtained with four clones from a second B27.05-positive donor RT (data not shown). Furthermore all 15 B27.02-positive LY clones tested, whether they displayed subtype-specific restriction (e.g., LYc21) or B27.02/B27.05 restriction (e.g., LYc38), also mapped to the EBNA3C target antigen. Assays on 35 B27.04-positive DH clones produced two patterns of results. Four clones (e.g., DHc1) again mapped to EBNA3C, whereas another 31 clones (e.g., DHc50) recognized a different viral protein, LMP2.

Identification of B27-restricted Peptide Epitopes in EBNA3C. Since EBNA3C appeared to be a common target protein for CTL responses restricted through three different B27 subtypes, we sought to determine the epitope specificity of these responses using synthetic peptides. In an initial series of experiments, we screened a panel of 15-mer peptides (each overlapping by 10 amino acids and spanning the entire 938 amino acid sequence of EBNA3C) for their ability to sensitize autologous PHA blast targets to CTL recognition. Because of the requirement for large numbers of effectors, these assays used polyclonal CTL preparations from B27 subtyped donors that were known to contain a dominant B27-restricted component and to recognize EBNA3C by recombinant vaccinia

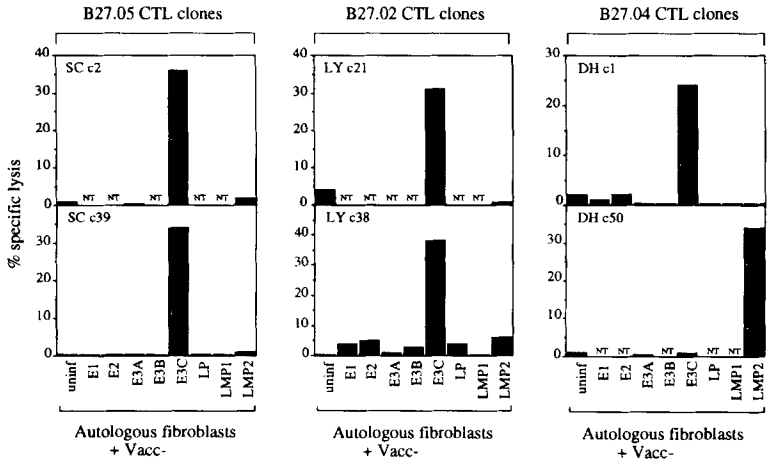

Figure 2. Analysis of EBV target antigens recognized by the same representative B27-restricted CTL clones from donors SC, LY, and DH as used in Fig. 1. Individual clones were tested against autologous fibroblasts either uninfected (*uninf*) or preinfected with recombinant vaccinia viruses (*Vacc-*) expressing the individual EBV latent proteins EBNAs 1, 2, 3A, 3B, 3C, -LP, and LMPs 1 and 2. E/T ratios were between 2:1 and 8:1. Results are expressed as percent specific lysis and are illustrative of those seen in several repeated assays.

virus mapping (24). We found that CTL preparations of this kind from the B27.05-positive donor RT, the B27.02-positive donor LY, and the B27.04-positive donor DH all gave significant killing against one particular 15-mer peptide, EBNA3C residues 256-270 (data not shown).

The result was confirmed and extended in subsequent experiments where the CTL preparations were screened against an independent panel of 10-mer peptides representing all possible EBNA3C sequences with an arginine residue at position 2, i.e., all peptides satisfying the primary requirement of the B27.05 peptide motif (10). Fig. 3 shows the overall results expressed as mean target cell lysis ± SD from six to eight such assays per donor, in each case involving effector CTLs from at least three independent activations from peripheral blood. Significant levels of lysis above the general background were observed for two overlapping 10-mer peptides (EBNA3C residues 257-266 and 258-267) with all three effector populations, i.e., with B27.05-positive RT CTLs, with B27.02-positive LY CTLs and with B27.04-positive DH CTLs. Polyclonal CTLs from two other B27.05-positive donors, SC and EN, also showed reactivity only against the above two peptides (data not shown). Significantly, these overlapping sequences both lay within the particular 15-mer peptide (EBNA3C residues 256-270) identified in the initial screening experiments. None of the other 10-mer peptides gave reproducible lysis above background with any of the CTL preparations tested, with the single exception of EBNA3C residues 249-258. This sequence was recognized by some but not all polyclonal CTL preparations from the B27.05 donor RT, though not by any other donor.

Having used polyclonal CTLs in these initial peptide screening assays, we then tested the CTL clones for their ability to recognize the two 10-mer peptides 257-266 and 258-267 and the shared 9-mer 258-266 (sequence RRIYDLIEL). Of the B27-restricted clones that had been mapped to the EBNA3C target protein in recombinant vaccinia experiments, all of those that were still available for testing in peptide sensitization experiments proved to be specific for the 258-266 epitope. Fig. 4 shows the results obtained when the same representative panel of B27.05, B27.02, and B27.04-positive CTL clones used in Figs. 1 and 2 were assayed on PHA blasts of the three different B27 subtypes preexposed to EBNA3C peptides 257-266, 258-267, and 258-266. All five B27.05-positive SC clones tested in this way (e.g., SCc2) recognized the 258-266 epitope on B27.05-positive blasts but not on the other two subtypes. Furthermore, three CTL clones from the second B27.05-positive donor RT gave the same result (data not shown). Interestingly, all 13 B27.02-positive LY clones tested, whether entirely B27.02 restricted (e.g., LYc21) or B27.02/B27.05 restricted (e.g., LYc38) in the earlier analysis (see Fig. 1), were able to recognize the 258-266 epitope when presented on B27.02 and B27.05 blast cells. Finally, two B27.04-positive DH clones showing EBNA3C specificity (e.g., DHc1) likewise recognized the 258-266 epitope but only when presented on a B27.04 background. Included as controls in the above experiment were three other EBNA3C peptides (79-88, 254-263, and 255-264) whose sequences matched the B27.05 peptide motif as closely as the 258–266 epitope itself. These peptides never mediated significant lysis by B27-restricted effectors.

We also had access to a limited number of cryopreserved lymphocytes from a second B27.04-positive donor, DW, who in earlier studies had been found to generate a B27.04-restricted EBV-specific CTL response (28). Cloning experiments from this lymphocyte sample yielded a single B27.04-restricted CTL clone and this also mapped to the same EBNA3C 258-266

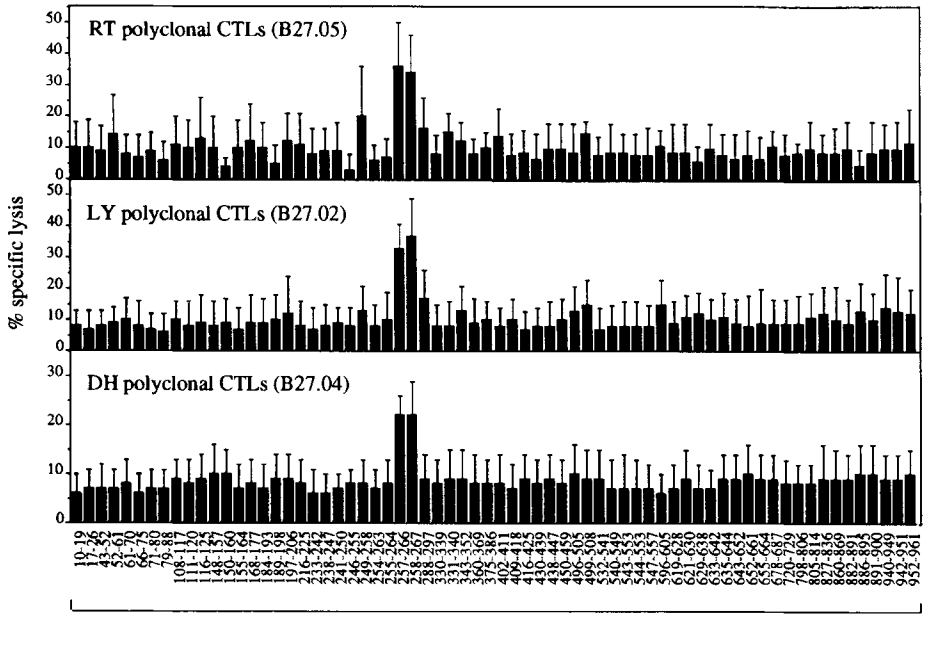

Figure 3. Peptide epitopes recognized by EBNA3C-specific, B27-restricted polyclonal CTLs. Selected 10-mer peptides from the EBNA3C sequence were screened for their ability to sensitize PHA blast targets to autologous EBV-specific polyclonal CTL preparations from the B27.05-positive donor RT (A2, A24; *B27.05*, B35), from the B27.02-positive donor LY (A1, A24; *B27.02*, B35), and from the B27.04-positive donor DH (A2, A11; *B27.04*, B40). Results are a compilation of data from three independent polyclonal CTL activations from each donor and are expressed as the mean percent specific lysis (±SD) observed in a total of six to eight replicate assays per donor. E/T ratios were between 5:1 and 15:1. For all three CTL donors, average levels of background lysis up to 10% were observed for control PHA blast targets not exposed to peptides. Peptides are identified by numbers indicating their position in the EBNA3C primary sequence (42). The panel includes all possible EBNA3C peptides with an arginine residue at position 2.

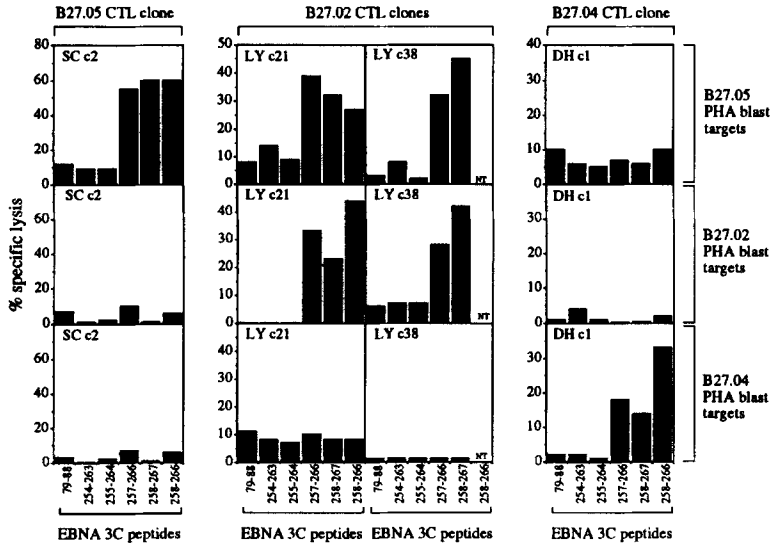

Figure 4. Peptide epitopes recognized by EBNA3C-specific, B27-restricted CTL clones. Representative B27-restricted, EBNA3C-specific CTL clones from donors SC, LY, and DH (as in Fig. 1) were tested against the 10-mer peptides 257-266, 258-267, and their shared 9-mer 258-266. Other 10-mer peptides 79-88, 254-263, and 255-264 were included as negative controls. These peptides (along with the 258-266 sequence) show the best correlations within EBNA3C with the proposed B27.05 peptide motif (10). All CTL clones were tested on PHA blast targets representing the three B27 subtypes, B27.05, B27.02, and B27.04. E/T ratios were between 2:1 and 8:1. Results are expressed as percent specific lysis and are illustrative of those seen in several repeated assays.

peptide when screened on PHA blast targets from the B27.04-matched donor DH (data not shown). A summary of results from the analysis of EBNA3C-specific CTL responses in B27-positive donors is presented in Table 1.

Identification of B27.04-restricted Epitope in LMP2. A final series of experiments sought to determine the peptide epitope specificity of the B27.04-restricted DH CTL clones that had recognized LMP2 rather than EBNA3C in the original recombinant vaccinia experiments (see Fig. 2). In this case, we screened all available clones against a panel of 16 selected peptides from the LMP2 sequence, namely all possible 9-mers with an arginine residue at position 2. All 17 DH clones tested in this way showed clear reactivity against a single 9-mer representing LMP2 residues 236-244 (sequence RRRWRRLTV); none of the other LMP2 peptides were recognized. Data from two representative clones (DHc37, DHc50) are shown in Fig. 5 from assays in which the LMP2 9-mer peptides were presented on autologous B27.04-positive PHA blasts and also on B27.05 and B27.02-positive PHA blasts. The B27.04 restriction apparent in earlier experiments on LCL targets was again in evidence, reactivity against the cognate peptide 236-244 only being observed on B27.04-positive target cells. This LMP2 epitope is included in the overall summary of B27-restricted CTL responses shown in Table 1.

Discussion

The existence of allelic polymorphism within the serologically defined HLA-B27 family was first highlighted by work on alloreactive and virus-specific CTL responses (29–32), and many of the different subtype molecules thus identified proved to be distinguishable in isoelectric focusing studies (33, 34). The ability of T cells to discriminate between targets expressing different B27 subtypes is best documented for allo-CTL clones,

Table 1. *Summary of EBV-coded CTL Epitopes Presented by B27 Subtypes*

Donor	B27 Subtype	Effector CTLs	Epitope location	Epitope sequence
SC	27.05	Polyclonal + clones	EBNA3C 258-266	RRIYDLIEL
EN	27.05	Polyclonal	EBNA3C 258-266	RRIYDLIEL
RT	27.05	Polyclonal + clones	EBNA3C 258-266	RRIYDLIEL
		Polyclonal	EBNA3C 249-258	LRGKWQRRYR*
LY	27.02	Polyclonal + clones	EBNA3C 258-266	RRIYDLIEL
DW	27.04	CTL clone	EBNA3C 258-266	RRIYDLIEL
DH	27.04	Polyclonal + clones	EBNA3C 258-266	RRIYDLIEL
		CTL clones	LMP2 236-244	RRRWRRLTV

* Minimal epitope not yet defined.

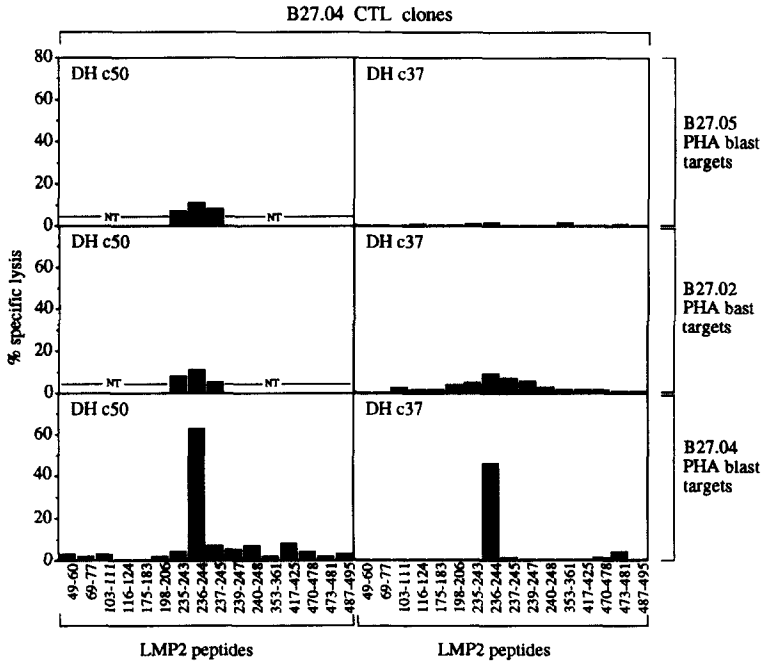

Figure 5. Peptide epitopes recognized by LMP2-specific, B27.04-restricted CTL clones. Representative B27.04-restricted, LMP2-specific CTL clones from donor DH (A2, A11, B27.04, B40) were tested on all possible 9-mer peptides from the LMP2 sequence containing an arginine residue at position 2. Peptides are again identified by their position in the LMP2 primary sequence (43). Clones were tested on PHA blast targets representing the three B27 subtypes, B27.05, B27.02, and B27.04. E/T ratios were between 4:1 and 8:1. Results are expressed as percent specific lysis and are illustrative of those seen in several repeated assays.

and by these criteria, none of the natural B27 mutations is immunologically silent (32; for a review see reference 11). Work to date on B27 molecules as restriction elements for antiviral CTL responses leads to a similar conclusion (29–31), although some crossrecognition between B27.05 and B27.02 (35) and between B27.05 and B27.03 (36) has been reported using polyclonal influenza-specific CTL populations. Throughout the above studies, however, there was always a very clear distinction between the B27.01/B27.02/B27.05 group and the B27.04/B27.06 group of alleles, apparently reflecting a key role for the identity of a polymorphic residue at position 152 in the α_2 helix of the peptide binding groove (32). In the present work, we have used as probes EBV-specific CTL clones from B27.05-, B27.02-, and B27.04-positive donors. As shown in Fig. 1, the majority of such cells were subtype specific in their restriction, the only exception being a subset of B27.02-positive clones that could recognize EBV in the context of B27.05 as well as B27.02. The effectors in this viral system are therefore at least as sensitive to B27 subtype polymorphism as allospecific or influenza-specific CTLs. This established, we went on to examine the EBV antigen specificity of these subtype-restricted responses.

Some of the CTL donors used here (SC and RT, B27.05; LY, B27.02; DH, B27.04) had been subjects in an earlier survey (24) where the dominant B27-restricted component within polyclonal CTL populations had mapped to the viral nuclear antigen EBNA3C. In the present work, all of the B27.05-positive, all of the B27.02-positive, and a subset of the B27.04-positive CTL clones established from these same donors likewise mapped to EBNA3C (Fig. 2). Detailed analysis of the above responses at the peptide level gave striking evidence of epitope sharing between the different B27 subtypes. Thus polyclonal and/or clonal CTLs from three B27.05-positive donors, RT, SC, and EN, from one B27.02 positive donor LY, and from two B27.04-positive donors DH and DW, all recognized the same peptide EBNA3C residues 258-266. This clearly must be the immunodominant epitope within EBNA3C since it was identified not only from a selected panel of 10-mer peptides representing all those sequences that satisfied the primary requirement for B27.05 binding, namely an arginine at position 2, but also from a complete panel of EBNA3C 15-mer synthesized without any bias towards the B27.05 peptide motif. Note that these screening assays were conducted at relatively high peptide concentrations, in the 10^{-5}–10^{-6} M range, in order to identify all possible CTL epitopes of whatever affinity (37). However, more recent experiments (data not shown) indicate that the 258-266 peptide can effectively sensitize B27 targets to CTL lysis at concentrations down to at least 10^{-9} M, again emphasizing the efficiency with which this epitope is recognized.

The epitope sequence RRIYDLIEL conforms well to the proposed B27.05 peptide motif in having an arginine at position 2, a positively charged amino acid at position 1, and a hydrophobic residue at position 3. The presence of a hydrophobic residue at position 9, rather than the preferred positively charged amino acid, has been noted in certain other B27.05-associated peptides (10). The ability of this peptide to bind and be presented as an immunodominant CTL epitope not only by B27.05, but also by B27.02 and B27.04 is very significant in view of the known differences between these subtypes in the structure of their peptide binding grooves (11). On the one hand, conservation of the unique B pocket throughout the B27 family of alleles strongly suggests that the absolute requirement for arginine at position 2 will be maintained in all B27-binding peptides (38). On the other hand, relative to B27.05, there are amino acid changes in

B27.02 (affecting positions 77, 80, and 81 in the α1 helix of the groove) and in B27.04 (affecting positions 77 in the α1 helix and 152 in the α2 helix) which would be anticipated to alter peptide/HLA contacts involving residues in the COOH-terminal region of the peptide. From the CTL data, however, it is clear that these subtype changes do not preclude binding of the RRIYDLIEL sequence. In fact, molecular modeling of the B27.05, B27.02, and B27.04 peptide binding grooves suggests that this EBNA3C peptide is ligated in an almost identical manner in all three situations (Thorpe, C. J., D. S. Moss, J. M. Brooks, A. B. Rickinson, and P. J. Travers, manuscript in preparation), with the effects of B27 subtype polymorphism being largely negated by movement within the bed of water molecules known to be present within the B27.05 crystal structure (9).

The overall structures of these B27–EBNA3C peptide complexes must nevertheless be subtly different since they can be distinguished by TCR molecules. Thus both B27.05-positive CTL clones and B27.04-positive CTL clones only recognize target cells expressing their own B27 subtype. This is true whether the peptide epitope is generated in target cells from an endogenously expressed EBNA3C protein or is provided exogenously as synthetic peptide (Figs. 1 and 4). Interestingly, on LCL targets, some B27.02-positive CTL clones were capable of recognizing the B27.05 subtype, whereas other clones were entirely B27.02 restricted (Fig. 1). However, when optimal concentrations of peptide were supplied exogenously, both sets of clones were able to recognize B27.02 and B27.05 targets (Fig. 4). In a similar way, overexpression of EBNA3C from a recombinant vaccinia virus in B27.05-positive fibroblasts also allowed crossrecognition by CTL clones such as LYc21 which display B27.02 restriction on LCL targets (data not shown). We presume that such crossrecognition involves a low affinity interaction that requires higher numbers of B27–peptide complexes to be presented on the cell surface before target cell lysis can be achieved.

The identification of an additional B27.04-restricted CTL epitope, which is apparently not recognized by B27.05- or B27.02-positive donors, is very interesting. Thus, whereas the dominant EBV-specific reactivity within polyclonal CTL preparations from the B27.04-positive donor DH was against the EBNA3C epitope (24; Fig. 3, and data not shown), several independent clonings from this donor yielded a majority of CTL clones mapping to an epitope within LMP2. The sequence of this LMP2 epitope, RRRWRRLTV, is on the one hand compatible with the proposed B27.05 peptide motif at conserved positions 1, 2, and 9, but incompatible at position 3 where arginine replaces the expected hydrophobic residue. Molecular modeling indicates that this LMP2 peptide can indeed be stably accommodated within the peptide binding groove of B27.04, but not of B27.02 or B27.05. This reflects the importance of the valine to glutamate substitution at position 152 in the B27.04 groove, which allows hydrogen bonding between the glutamate side chain and guanidinium groups in the side chains of arginine residues at positions 3 and 5 in the LMP2 peptide (Thorpe, C. J., et al., manuscript in preparation). The presence of a valine residue at position 152 in B27.02 and B27.05 molecules precludes this type of interaction. We would suggest therefore that all three subtypes can bind peptides strictly adhering to the proposed B27.05 motif, but that B27.04 can in addition bind variants that contain arginine at positions 3 and 5.

The present results are particularly significant in relation to the very strong linkage that exists between the HLA-B27 family of alleles and ankylosing spondylitis. Thus, it has been argued that CTL responses to an arthritogenic peptide presented only by B27 molecules might underlie disease pathogenesis. This implies that all the B27 subtypes known to be disease linked (B27.01, .02, .04, .05, and .06) must be capable of presenting the same peptide. Before the present study, the only evidence for such epitope sharing between B27 subtypes was indirect and came from crossrecognition by rare allospecific CTL clones where the identity of the presumed peptide epitope was not known (39). Here we show in a model system, studying CTL responses to a known human pathogen EBV, that at least three of the above B27 subtypes (B27.02, .04, and .05) do indeed present the same immunodominant viral peptide. Such convergence is remarkable given the extensive coding potential of this virus and the range of proteins against which, on other HLA backgrounds, EBV-induced CTL responses can be directed (24, 40). In light of these results, it will be interesting to extend the present approach to include CTL donors of other B27 subtypes. One might, for instance, anticipate similarities between B27.01 and the closely related B27.02 allele, and between B27.06 and the closely related B27.04 allele (11). It will be particularly important to analyze CTL epitope choice by the B27.03 subtype which, though closely related to B27.05, may not show the same disease linkage (41).

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