The Same Natural Ligand Is Involved in Allorecognition of Multiple HLA-B27 Subtypes by a Single T Cell Clone: Role of Peptide and the MHC Molecule in Alloreactivity

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The Same Natural Ligand Is Involved in Allorecognition of Multiple HLA-B27 Subtypes by a Single T Cell Clone: Role of Peptide and the MHC Molecule in Alloreactivity

Alberto Paradela,2* Marina García-Peydro,2* Jesús Vázquez,*, Didier Rognan,† and José A. López de Castro3*

The human alloreactive CTL clone 27S69, raised against B*2705, cross-reacts with B*2702 and B*2703, but not with B*2701, B*2704, B*2706, or B*2710. Its natural epitope was identified by electrospray/ion trap mass spectrometry, as the proteasome-derived RRFPYYVYV octamer. This is the first HLA-B27 ligand shown to be immunogenic in alloreactivity. The RRFPYYVYV nonamer, also found in the B*2705-bound peptide pool, was recognized much less efficiently, demonstrating that an alloreactive CTL distinguishes between very similar natural ligands. Molecular modeling suggested that this was due to the different conformation of each peptide in complex with B*2705. B*2702- and B*2703-RMA-S cells were lysed by CTL 27S69 when sensitized with the octamer, demonstrating that cross-reaction with these subtypes is through recognition of the same peptide as in B*2705. B*2704-, B*2706-, and B*2710-RMA-S cells were not sensitized for lysis, in spite of efficient binding of the octamer, indicating that polymorphism in these subtypes directly impairs allorecognition. B*2701-RMA-S and -C1R cells were sensitized for lysis by the octamer, suggesting lack of the endogenous peptide epitope on this subtype. Absence of the octamer in the B*2701-bound peptide pool further suggested that B*2701 polymorphism impairs the generation of this peptide.

and demonstrate the contribution of polymorphic HLA-B27 residues to the allospecific epitope. The same CTL clone distinguished between its peptide epitope and another closely related natural HLA-B27 ligand. The structure of the allospecific epitope and the molecular basis for this discrimination were analyzed by molecular modeling.

**Materials and Methods**

**CTL 27S69**

The anti-B*2705 alloreactive CTL 27S69 clone and its culture conditions have been described (20). It was raised from donor SR (HLA-A3, -B7, -DR2, 7) by in vitro stimulation with LCL B*2705 (HLA-A3, -B7, -DR3, 5). Besides B*2705, CTL 27S69 recognized B*2702, B*2703, and HLA-B61, but not B*2701, B*2704, or B*2706, as established with HLA-typed LCL.

**HLA-B27 transfectant cell lines**

Hmy2-C1R (C1R) is a human lymphoid cell line with low expression of its endogenous class I Ags. These cells and their transfectants expressing HLA-B27 subtypes were cultured in DMEM (Life Technologies, Paisley, UK) containing 5% heat-inactivated FCS. T2 is a TAP-deficient human cell line of lymphoid origin (23). The B*2705-T2 transfectant was a kind gift of Dr. David Yu (University of California, Los Angeles, CA). It was cultured in DMEM supplemented with 5% FCS. RMA-S is a TAP-deficient mutant cell line derived from the murine H-2^d^ lymphoma RBL-5. These cells and their transfectants expressing HLA-B27 subtypes plus human β2-microglobulin (6, 24–26) were cultured in RPMI 1640 supplemented with 10% FCS. When cultured at 26°C, T2 and RMA-S cells express class I molecules presumably devoid of peptides. These molecules are unstable at 37°C, but their surface expression at this temperature can be stabilized by exogenous peptide ligands.

**Isolation of HLA-B27-bound peptides**

About 1–1.5 × 10^7^ B*2705-C1R cells were lysed at 4°C in 20 mM Tris-HCl buffer, 150 mM NaCl, 1% Nonidet P-40, pH 7.5, containing the following protease inhibitors: 10 μg/ml leupeptin, 2 μg/ml pepstatin, 2.5 μg/ml aprotinin (all from Boehringer Mannheim, Mannheim, Germany), 18.5 μg/ml aprotinin, 1 mM EDTA, 2 mM PMSF, 258 μg/ml 1,10 phenantroline (Sigma, St. Louis, MO), and 0.2% sodium azide. Cell lysates were centrifuged, and the supernatant was filtered, precleared through a CNBr-activated Sepharose 4B column (Pharmacia, Uppsala, Sweden), and subjected to affinity chromatography using the W6/32 mAb (IgG2a, specific for a monomorphic HLA-A, -B, and -C determinant). HLA-B27–binding peptides were eluted from the column with 0.1% TFA in water at 37°C, but their surface expression at this temperature can be stabilized by exogenous peptide ligands.

**Mass spectrometry analysis and peptide sequencing**

The peptide composition of individual HPLC fractions was determined by matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry. A calibrated reflex instrument (Brucker-Franzen Analytik, Bremen, Germany) operating in the positive ion reflection mode was used. Five-microliter aliquots of a given HPLC fraction were dried, resuspended in 2 μl of 0.1% TFA in water:acetoneitrile, 2:1, and mixed with 2 μl of saturated o-cyano-4-hydroxycinnamic acid matrix in 0.1% TFA in water:acetoneitrile ratio of 2:1. One microliter of the mixture was dried and subjected to analysis.

Peptide sequencing was conducted in a LCQ electrospray/ion trap mass spectrometer (Finnigan MAT, San Jose, CA), equipped with a microspray probe. HPLC fractions were dried down and resuspended in 5 μl methanol/water (1:1) containing 0.1% formic acid. One microliter of this solution was used for analysis. Accurate peptide mass and charge of ionic species were determined by performing "Zoomscan" spectra. This is a high-reso-

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about 1010 C1R transfectant cells was fractionated by HPLC (Fig. 1). The elution positions of fractions 205–208, which sensitized B*2705-T2 cells for lysis by CTL 27S69, are indicated by an arrow. B, Lysis of B*2705-T2 target cells sensitized with 8-μl aliquots of HPLC fractions of the B*2705-bound peptide pool by CTL 27S69. Specific lysis of the control B*2705-C1R cells at the E:T ratio used (2:1) was 72%. Experimental conditions are described in Materials and Methods.

FIGURE 1. A, HPLC fractionation of the B*2705-bound peptide pool from B*2705-C1R cells. Only the peptide portion of the chromatogram is shown. The elution positions of fractions 205–208, which sensitized B*2705-T2 cells for lysis by CTL 27S69, are indicated by an arrow. B, Lysis of B*2705-T2 target cells sensitized with 8-μl aliquots of HPLC fractions of the B*2705-bound peptide pool by CTL 27S69. Specific lysis of the control B*2705-C1R cells at the E:T ratio used (2:1) was 72%. Experimental conditions are described in Materials and Methods.

Results

Identification of the peptide epitope recognized by CTL 27S69

The peptide dependency of CTL 27S69 was suggested by failure to lyse B*2705-T2 cells. In a first attempt to determine the epitope recognized by this CTL, the B*2705-bound peptide pool from about 10^10 C1R transfectant cells was fractionated by HPLC (Fig. 1A). Aliquots of individual fractions were incubated with B*2705-T2 transfectants, and tested for lysis by the CTL clone. A peak of sensitizing activity spanning HPLC fractions 205–208 (Fig. 1B) suggested that CTL 27S69 recognized a peptide eluting in these fractions. Thus, their composition was determined by MALDI-TOF mass spectrometry. Fractions 205 and 206, which showed the highest sensitizing activity, contained at least 15 and 7 molecular species, respectively. The amino acid sequences of two of the four peptides that eluted in both of these fractions were determined by electrospray/ion trap mass spectrometry. The sequence of the RRFFPYVY octamer (molecular mass: 1149 Da), a proteasome C5 subunit-derived peptide, was determined from its MALDI-TOF mass spectra of all of the HPLC fractions containing the octamer efficiently sensitized B*2705-T2 (Fig. 3A) and the RRFFPYVY octamer was quantitatively analyzed. The octamer efficiently sensitized B*2705-T2 (Fig. 3A) and B*2705-RMA-S (Fig. 3B) cells for lysis: half-maximal lysis was obtained at 1.8 × 10^-8 M and 1.4 × 10^-7 M, respectively. This difference is consistent with the lower avidity of human CTL for murine, relative to human target cells. The nonamer was recognized about 100- or 1000-fold less efficiently on B*2705-T2 and B*2705-RMA-S targets, respectively. This was not due to lower binding of the nonamer: actually, this peptide bound better than the octamer to B*2705-RMA-S cells (Fig. 3C). The nonamer was about four-fold more abundant than the octamer in the B*2705-bound peptide pool, as estimated from the respective intensity peaks in the MALDI-TOF mass spectra of all of the HPLC fractions containing either peptide. This correlates with their relative binding to B*2705 in vitro.
Taken together, these results indicate that CTL 27S69 discriminates between the RRFFPYYV octamer and its C-terminally extended RRFFPYYVY nonamer, in a way unrelated to the relative binding or abundance of both peptides.

CTL 27S69 cross-reacts with B*2702 and B*2703 through recognition of the same peptide as in B*2705.

CTL 27S69 cross-reacts with B*2702 and B*2703 on LCL (20). Thus, binding of the RRFFPYYV octamer and the corresponding nonamer, and their recognition in the context of these two subtypes were tested. Both peptides bound to B*2702 (Fig. 4A) and B*2703 (Fig. 4B), with EC<sub>50</sub> values in the range commonly found among natural ligands. Indeed, the nonamer was sequenced from the B*2703-bound peptide pool (38). In addition, CTL 27S69 efficiently killed B*2702-RMA-S targets sensitized with the octamer (half-maximal lysis at 0.8 × 10<sup>-7</sup> M) and, about 70-fold molar less efficiently, with the nonamer (Fig. 4C). The octamer, but not the nonamer, was also recognized in the context of B*2703 (half-maximal lysis at 4.6 × 10<sup>-7</sup> M) (Fig. 4D).

These results indicate that the RRFFPYYV octamer binds to B*2702 and B*2703 with an affinity compatible with its binding in vivo to the two subtypes, and that cross-reaction of CTL 27S69 with B*2702 and B*2703 is through recognizing the same peptide as in B*2705.

B*2704, B*2706, and B*2710 bind, but do not present, the peptide epitope to CTL 27S69

This CTL clone failed to lyse LCL expressing B*2704 or B*2706 (20), as well as B*2710-C1R cells (data not shown). All three subtypes have Glu<sub>152</sub>, instead of Val<sub>152</sub>, which is present in all other HLA-B27 subtypes. This is the only change between B*2710 and B*2705. Binding to B*2704-, B*2706-, or B*2710-RMA-S cells, and lysis of these transfectants in the presence of the peptide epitope were analyzed (Fig. 5). Both the RRFFPYYV octamer and the corresponding nonamer bound in vitro to B*2704, B*2706, and B*2710 with EC<sub>50</sub> values in the range of natural ligands (Fig. 5A–C). In addition, the RRFFPYYV octamer was identified and sequenced from a HPLC fraction of B*2704-bound peptides.
by electrospray/ion trap mass spectrometry (data not shown). In spite of the binding observed, CTL 27S69 failed to lyse B*2704-, B*2706-, or B*2710-RMA-S cells even at the highest peptide concentration used (Fig. 5D). The octamer also failed to sensitize C1R transfectants expressing these subtypes (data not shown).

**FIGURE 3.** A, Lysis of B*2705-T2 target cells sensitized with the synthetic RRFFPYV (●) and RRFFPPYYV (■) peptides by CTL 27S69. The B*2705 natural ligand RRYQKSTEL (▲) was used as negative control. Half-maximal lysis was obtained at $1.8 \times 10^{-8}$ M of the octamer. The same lysis required $2.5 \times 10^{-8}$ M of the nonamer. Specific lysis of B*2705-C1R cells at the E:T ratio used (0.5:1) was 49%. B, Lysis of B*2705-RMA-S target cells sensitized with RRFFPPYYV, RRFFPPYYVV, or the negative control RRYQKSTEL by CTL 27S69. Half-maximal lysis was obtained at $1.4 \times 10^{-7}$ M of the octamer. The same lysis required more than $10^{-6}$ M of the nonamer. Specific lysis of B*2705-C1R cells at the E:T ratio used (0.75:1) was 45%. C, Binding of RRFFPPYYV, RRFFPPYYVV, and KTGGPIYKR as negative control to B*2705 on RMA-S transfectant cells. EC$_{50}$ was 2 and 8 μM for the nonamer and the octamer, respectively. Data are means of two experiments. For experimental details, see Materials and Methods.

**FIGURE 4.** A, Binding of RRFFPYV (●), RRFFPPYYV (■), and KTGGPIYKR (▲) as negative control to B*2702 on RMA-S cells. EC$_{50}$ was 3 and 4 μM for the nonamer and the octamer, respectively. B, Binding of the same peptides to B*2703 on RMA-S cells. EC$_{50}$ was 6 and 5 μM for the nonamer and the octamer, respectively. Data for B*2702 and B*2703 are means of two experiments. C, Lysis of B*2702-RMA-S cells sensitized with the RRFFPYV and RRFFPPYYV peptides, by CTL 27S69. Half-maximal lysis was obtained at $0.8 \times 10^{-9}$ M of the octamer. The same lysis required $5.5 \times 10^{-8}$ M of the nonamer. Specific lysis of B*2702-C1R cells at the E:T ratio used (0.9:1) was 76%. Data are means of three experiments. D, Lysis of B*2703-RMA-S cells sensitized with the same peptides, by CTL 27S69. Half-maximal lysis was obtained at $4.6 \times 10^{-7}$ M of the octamer. Specific lysis of B*2703-C1R cells at the E:T ratio used (0.9:1) was 68%. Data are means of two experiments. For experimental details, see Materials and Methods.
These results indicate that lack of cross-reaction of CTL 27S69 with B*2704, B*2706, and B*2710 is not due to absence or inefficient binding of the corresponding peptide epitope, but to direct impairment of T cell recognition by structural features of these subtypes, including at least the Glu152 residue.

B*2701 presents exogenous, but not the endogenous peptide epitope to CTL 27S69

CTL 27S69 did not lyse B*2701-positive LCL (20), or B*2701-C1R transfectants. As in B*2705, the nonamer, which is a natural B*2701 ligand (26), bound better than the octamer to B*2701-RMA-S cells (Fig. 6A). Although the EC_{50} of the octamer was also in the range of natural ligands, it was not found in the corresponding HPLC fractions from the B*2701-bound peptide pool, upon analysis by electrospray/ion trap mass spectrometry. Other peptides coeluting with the octamer in the B*2705-bound peptide pool were also in the fractions analyzed from B*2701 (data not shown). This suggests that the octamer is not bound in vivo to B*2701.

Thus, failure of CTL 27S69 to cross-react with B*2701 LCL is probably due to absence of the peptide epitope on this subtype. In peptide sensitization assays, significant lysis of B*2701-RMA-S cells was obtained with the octamer (half-maximal lysis at 10^{-6} M) and, about 100-fold lower, with the nonamer (Fig. 6B). In addition, B*2701-C1R cells were highly sensitized with the octamer (half-maximal lysis at 1.7 × 10^{-8} M) (Fig. 6C). These results indicate that RRFPYYVY is efficiently recognized by CTL 27S69 in the context of B*2701, further supporting that lack of cross-reaction with this subtype is due to lack of constitutive binding of the peptide to B*2701 in vivo.

Molecular modeling of the allospecific epitope recognized by CTL 27S69

The molecular basis for the capacity of CTL 27S69 to distinguish between the RRFPYVV and RRFPYVVY ligands was addressed by molecular modeling of both peptides in complex with B*2705. They were stabilized in the peptide-binding groove by numerous hydrogen bonds and nonpolar interactions. However, whereas hydrogen bonding was quantitatively similar in both complexes, the nonamer established significantly more nonpolar contacts (total of 72) than the octamer (total of 53), mainly because of additional interactions of Tyr9 in the F pocket. The total buried surface areas for the octamer and the nonamer bound to B*2705 were 732 and 812 Å², respectively. These results are consistent with the better binding of the nonamer to B*2701 (Fig. 3).

The differential recognition of the octamer and nonamer by CTL 27S69 can be explained by the different conformations of both peptides in complex with B*2705 (Fig. 7). Although the accessible surface area was only slightly higher for the octamer (539 Å²) than for the nonamer (506 Å²), the conformation of the main chain was different, especially in the central part of the sequence (P4-P5),...
where it bulged out more prominently in the nonamer. Besides Arg1, which was partially accessible and similarly oriented in both peptides, three other side chains (Phe4, Pro5, and Tyr7) were accessible for the octamer, and four (Phe4, Pro5, Tyr7, and Val8) for the nonamer. Phe4 was rather similarly oriented in both cases. In contrast, Pro5 bulged out much more for the nonamer. In addition, a major qualitative difference occurred for Tyr7. In the nonamer, its side chain was located in a three-dimensional space not occupied by any atom of the bound octamer. Val8 was exposed for the nonamer. In the octamer, this residue is directed inward into the F pocket in the same way as Tyr9 in the nonamer, although the larger Tyr9 side chain gets deeper into this pocket.

In conclusion, molecular modeling provides an explanation for the moderate cross-reactivity between the octamer and the nonamer in complex with B*2705. Conserved contacts with the TCR might be provided by the B*2705 molecule, and by Arg1 and Phe4 in both peptides. The substantial differences in the conformation and/or accessibility of the Pro5, Tyr7, and Val8 side chains explain the weaker cross-reaction with the nonamer.

Discussion

Molecular studies on alloreactivity are hampered by the difficulty of identifying the peptides involved in allospecific T cell epitopes. A natural ligand specifically recognized by an alloreactive CTL clone raised against B*2705 was reported in this work. Aside from a peptide with an unclear relationship to the natural epitope (39), this is, to our knowledge, only the second naturally presented peptide identified as the epitope of an alloreactive CTL, and the first one from a known protein. It is also the first HLA-B27 ligand shown to be immunogenic in allospecific T cell responses. Its identification was possible through combining peptide sensitization of TAP-deficient target cells, and sequencing by electrospray/ion trap mass spectrometry. This is a powerful methodology, which adds to others, such as triple quadrupole (8, 40–42) and quadrupole/time of flight (43) electrospray mass spectrometry, as a suitable one for sequencing antigenic peptides eluted from class I MHC molecules.

The RRFFPYVV octamer derives from the C5 subunit of the proteasome, which is abundant in the cell (44), and was recovered from the B*2705-bound peptide pool in relatively substantial amounts. However, both abundant (9) and nonabundant (8, 11) peptides can stimulate alloreactive CTL. This epitope probably has high affinity for the TCR of CTL 27S69, since this clone can efficiently lyse murine B*2705-P815 (our unpublished results) and, in the presence of added peptide, RMA-S transfectant cells. Both TCR affinity and expression level of this peptide may contribute to its alloimmunogenicity. A minimum threshold of avidity is probably required to stimulate a given allo-CTL precursor. This threshold could be reached at low determinant density through high TCR affinity, or with high epitope expression in cases of lower affinity. Since serial TCR engagement by relatively few MHC-peptide complexes may be a mechanism of T cell triggering (45), nonabundant peptides may be allostimulatory. The requirements for alloimmunogenicity are important because they dictate the diversity of peptide epitopes in an allospecific T cell response. For instance, among the many class I-bound peptides involved in alloreactivity (1, 8), some can be immunodominant (13). The RRFFPYVV peptide was not recognized by three other anti-B*2705 clones tested, including one from the same donor (data not shown), but we cannot rule out that it might be one of a relatively limited set of peptides driving the anti-B*2705 response. This was suggested by restrictions in N+Db and Ja usage among B27-allospecific TCR (46).

Two aspects of this study provide novel insights into the molecular basis of T cell allorecognition. One is that another natural ligand closely related to the octameric epitope allowed us to assess the capacity of a CTL clone to distinguish between similar peptides in an allo-MHC context. The second aspect is the knowledge of the fine specificity of the CTL clone with HLA-B27 subtypes (20). This allowed us to analyze the relationship between expression and recognition of the peptide epitope in the context of different allo-MHC molecules, and therefore to assess the role of HLA polymorphism in allorecognition, aside from its effects on peptide binding.

Although self-restricted CTL are sensitive to subtle peptide changes, the capacity of alloreactive CTL to distinguish between related ligands could be limited by a more prominent contribution of MHC residues to the allospecific determinant. Cross-reaction of CTL 27S69 with the RRFFPYVV nonamer might be due, on the basis of modeling, to conservation of a portion of the epitope involving the MHC molecule and the peptideic Arg1 and Phe4. However, conformational and accessibility differences in the P5-P8 region explain the better recognition of the octamer. Thus, the
different alloantigenicity of these highly similar ligands illustrates
the critical role of peptide conformation in alloreactivity.
CTL cross-reacting with other MHC Ags do not necessarily rec-
ognize the same peptide in the various contexts. For example, the
murine alloreactive CTL clone 2C recognized three unrelated pep-
tides in the context of L^d, K^bm3, and, in self-restricted fashion, K^b,
respectively (9, 17, 18). Although CTL cross-reactions with HLA-
B27 subtypes were assumed to reflect similarities among subtype-
bound peptide repertoires (20, 47), recognition of the same peptide
by CTL 27S69 on B*2705, B*2702, and B*2703 provides the first

FIGURE 7. A, Overlay of the B*2705-
bound conformations of RRFFPYYV and
RRFFPYYVY. Backbone atoms of the
bound peptide are displayed as ribbon
tubes (cyan, RRFFPYYV; green, RRFF
PYYVY). Peptide side chains are repre-
sented by sticks with the following color
coding (blue, nitrogen; red, oxygen; cyan,
carbon atom of RRFFPYYV; green, carbon
atom of RRFFPYYVY). Ca atoms of the
peptides are labeled from the N terminus
(P1) to the C terminus (P8 or P9). The
B*2705 protein is not displayed. The figure
has been prepared using the MOLMOL
program (58). B, Side view, from the a2-
helix toward the a1-helix, of B*2705
(white) in complex with RRFFPYYV
(cyan). A TCR-a/b would bind diagonally
across the binding groove (22) at the top of
the figure. Both protein and peptide are rep-
resented by molecular surfaces, computed
and displayed by the GRASP program (59).
Only TCR-accessible peptide positions are
labeled. C, Side view of B*2705 (white) in
complex with RRFFPYYVY (green).
formal demonstration of this assumption. It also indicates that the changes between B*2702 or B*2703 with B*2705 (Y→H59 in B*2703; D→N77, T→A80, and L→A81 in B*2702) are not critical for this epitope. Presentation of the same viral peptide to B*2705-restricted CTL by B*2702 has been shown for EBV-specific T cells (48, 49). However, 7 of 10 B*2705-restricted CTL clones failed to recognize the viral epitope in the context of B*2702 (49). Thus, although not for CTL 27S69, polymorphic B*2702 residues can alter some T cell epitopes without impairing binding of the corresponding peptide.

From the results with B*2702 and B*2703, it should not be concluded that, upon expression of the relevant peptide, the structure of the alloantigen is not critical for allore cognition. Indeed, in spite of good binding, the RRRFPYYV octamer was not recognized in the B*2704, B*2706, or B*2710 context. Direct impairment of TCR binding by the E152 residue in these three subtypes is strongly supported by the fact that this is the only change between B*2710 and B*2705. We have reported recently that, in spite of little cross-reaction of anti-B*2705 CTL with B*2710, both subtypes bind in vivo similar peptide repertoire. Molecular modeling further suggested that the E152 change in B*2710 did not alter the conformation of bound peptides, but directly impaired TCR interaction (35). CTL 27S69 now provides the first example of an anti-B27 alloreceptive CTL clone that fails to recognize its peptide epitope across the V→E152 change. Therefore, besides the peptide, the structure of HLA-B27 directly and critically contributes to the allospecific epitope. The contribution of the MHC molecule to allore cognition is likely to severely limit cross-reactivity between class I alloantigens that bind common peptides (50).

Cross-reaction of CTL 27S69 with B*2701 only in the presence of exogenous peptide strongly suggests that the octamer epitope is not endogenously presented by this subtype, in spite of significant binding in vitro. This was supported by failure to detect the octamer in the B*2701-bound peptide pool by mass spectrometry. Since B27-bound peptides were isolated from C1R transfectants both for B*2701 and other subtypes, it is extremely unlikely that the octamer is not generated in B*2701-C1R transfectants due to a defect of these cells, independently of B*2701. Since the octamer bound similarly in vitro to B*2701 and B*2705 (EC09 7 and 8 μM, respectively), it also seems unlikely that failure to bind in vivo could be due to disadvantageous competition with other ligands for binding to B*2701, relative to B*2705. A possibility that we favor is that the octamer might be generated by trimming of the HLA-B27-bound nonamer, rather than by proteasome-mediated cleavage, and that in B*2701 such trimming is impaired. This is suggested by a report that MHC class I molecules influence the precise structure of endogenous ligands (51). Since the RRFPYYVYV nonamer is naturally presented by B*2701 (26), lack of cross-reaction with B*2701 indicates that the avidity of CTL 27S69 for the constitutive B*2701 + nonamer complex is insufficient for lysis.

In conclusion, identification of a peptide epitope involved in HLA-B27 allore cognition allowed us to establish that T cell allore cognition is very dependent both on the precise structure of the peptide, as to discriminate between closely related natural ligands, and on the structure of the MHC molecule. This may either influence expression of the peptide at the cell surface, or directly impair TCR binding.

The findings reported in this work have implications for the pathogenic role of HLA-B27 in ankylosing spondylitis and other spondyloarthropathies. Among other possible mechanisms, the arthritogenic peptide hypothesis proposes that peptide(s) presented by HLA-B27 would be recognized by autoreactive CTL activated upon external challenge, such as a bacterial infection (52). This hypothesis must explain that multiple subtypes, such as B*2705, B*2702, and B*2704, are associated with ankylosing spondylitis (53), whereas B*2706 and B*2709 are less or not associated with this disease (54, 55). Presumably, disease-associated subtypes should present some common peptides to CTL. Our results demonstrate that B*2705 and B*2702 present the same peptide to an alloreactive CTL, as they do to some EBV-restricted CTL clones (49). A potential problem arises from the fact that B*2704, although it binds some of the same ligands as B*2705 and B*2702 (25, 56), cannot present those that have been tested to the same CTL, as shown in this work and for EBV epitopes (48, 49). Cross-allo cognition of some anti-B*2705 alloreceptive CTL clones with B*2704 or with B*2704 plus B*2702, respectively (20), suggests that, in spite of residue 152, some peptides might be presented by all three B*2705, B*2702, and B*2704 subtypes to the same CTL. However, this awaits molecular identification of the corresponding peptide epitopes. There are at least two possibilities that make compatible the peptide-presenting properties of these three subtypes with their association to spondyloarthropathy. One is that putative arthritogenic peptides may be different for different subtypes. A second possibility, that might seem more likely, is that the same peptide is recognized in the context of different subtypes by arthritogenic CTL.

Although, obviously, alloreactive CTL are not related to B27-mediated spondyloarthropathy, their recognition of a same peptide in the context of different subtypes illustrates the extent to which HLA-B27 subtypes may interchangeably act as restriction elements for given peptides. This feature would be critical for a putative pathogenetic role of HLA-B27 as a peptide-presenting molecule.

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References

A NATURAL PEPTIDE LIGAND INVOLVED IN HLA-B27 ALLOREACTIVITY

      reactive CD8+ T cells can recognize unusual, rare, and unique processed pep-

      crystal structures of two viral peptides in complex with murine MHC class

      structures of two viral peptides in complex with murine MHC class

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