Identification of Novel HLA-B27 Ligands Derived from Polymorphic Regions of Its Own or Other Class I Molecules Based on Direct Generation by 20 S Proteasome*

HLA-B27 is strongly associated with ankylosing spondylitis. Natural HLA-B27 ligands derived from polymorphic regions of its own or other class I HLA molecules might be involved in autoimmunity or provide diversity among HLA-B27-bound peptide repertoires from individuals. In particular, an 11-mer spanning residues 169–179 is a natural HLA-B27 ligand with homology to proteins from Gram-negative bacteria. Proteasomal digestion of synthetic substrates demonstrated direct generation of the B27-(169–179) ligand. Cleavage after residue 181 generated a B27-(169–181)-13-mer that was subsequently found as a natural ligand of B*2705 and B*2704. Its binding to HLA-B27 subtypes in vivo correlated better than B27-(169–179) with association to spondyloarthropathy. Proteasomal cleavage generated also a peptide spanning B*2705 residues 150–158. This region is polymorphic among HLA-B27 subtypes and class I HLA antigens. The peptide was a natural B*2704 ligand. Since this subtype differs from B*2705 at residue 152, it was concluded that the ligand arose from HLA-B*3503, synthesized in the cells used as a source for B*2704-bound peptides. Thus, polymorphic HLA-B27 ligands derived from HLA-B27 or other class I molecules are directly produced by the 20 S proteasome in vitro, and this can be used for identification of such ligands in the constitutive HLA-B27-bound peptide pool.

Class I MHC molecules constitutively bind peptides, mainly of about 8–12 residues, which result from proteasomal degradation of endogenous proteins. Peptides are transported into the endoplasmic reticulum (ER) by means of the TAP (transporter associated with antigen processing) transporter and bind to nascent class I molecules in a process assisted by tapasin and other chaperones (1). The class I molecule, composed of the MHC heavy chain, β2m, and peptide, is then exported to the cell surface where it can be recognized by cytolytic T lymphocytes.

Proteasomes are multicatalytic complexes located in the nucleus and cytosol. In eukaryotic cells their catalytic core, or 20 S proteasome, consists of 28 subunits organized in heptameric sets to build a four-ring barrel structure. Each of the external rings contains seven noncatalytic α subunits, whereas each of the internal rings contains seven β subunits, three of which, β1, β2 and β5, are catalytic (2). In vertebrates these subunits can be cooperatively replaced by homologous interferon-γ-induced subunits β1i, β2i, and β5i, to form the immunoproteasome (3). The 20 S catalytic core, when bound to the PA700 (19 S) activator, results in the 26 S proteasome, which is involved in ATP-dependent digestion of ubiquitinated proteins (4–6).

The 20 S proteasome can also interact with the PA28 (11 S) regulator, which increases dual cleavage of polypeptide chains (7–10) and antigen presentation (11). The 20 S proteasome exhibits several protease activities. Some of these appear to be associated with a single subunit: trypsin-like with β2, chymotrypsin-like with β5, and postglutamyl activity with β1 (12). The other two activities, a “branched chain amino acid-prefering” and a “small neutral amino acid-prefering,” are not associated with a single subunit (13, 14). Cleavage specificity is modulated by amino acid residues in the vicinity of cleavable peptide bonds (15–19).

Although proteasomes are the major proteases involved in generation of MHC class I-bound peptides, the precise processing of these ligands remains unclear. An important issue is whether the proteasome directly generates the MHC ligands or peptide precursors that are subjected to further trimming. Direct generation of class I ligands has been reported previously (20–24), but there is also evidence for exopeptidase activity both in the cytosol and the ER (25–29). It has been suggested that proteasomes tend to generate the exact C-terminal ends of class I ligands, but are less precise at the N terminus, thus generating N-terminally extended precursors (30, 31). Another issue is that proteasomes may cleave peptide bonds internal to the sequence of natural ligands, leading to their destruction (18, 32, 33). The balance between cleavage leading to generation or destruction of a given peptide may determine its presence or influence its amount in the class I-bound pool.

HLA-B27 has special interest for its strong association with ankylosing spondylitis (AS) and reactive arthritis (ReA) (34, 35). Gram-negative bacteria, including species of Salmonella, Yersinia, Chlamydia, and Campylobacter, are known pathogenic agents for ReA (36). The mechanism involving HLA-B27 and bacteria in this disease is unknown. A classical hypothesis invoked molecular mimicry between bacterial and self-peptides

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presented by HLA-B27 as a source of a B27-directed autoreactive cytolytic T lymphocytes response that would be a primary pathogenetic event (37). Alternative mechanisms remain open (38–42). Although more than 90% of patients with AS and about 70% of those with ReA are B27-positive, most HLA-B27-positive individuals remain healthy. Additional genetic factors modulate susceptibility to these diseases (43), but their identity remains unknown.

This study addressed the generation of HLA-B27 ligands derived from HLA-B27 or other class I heavy chains by the 20 S proteasome. Misfolded class I polypeptides are dislocated to the cytosol (44, 45) and degraded by proteasomes (46), and HLA-B27 seems to misfold more than other HLA class I proteins (39). However, it is not known what peptides derived from class I molecules are HLA-B27 ligands, whether these peptides are directly produced by the proteasome, or whether proteasomal cleavage of class I molecules can be used to predict novel natural ligands of HLA-B27. To address these issues we focused on two regions of the a2 domain: around residues 169–179 and around residues 150–158. The first region has homology with protein sequences from Gram-negative bacteria (47). It was postulated that molecular mimicry between bacterial proteins and a peptide derived from this region of the HLA-B27 molecule and presented by HLA-B27 could elicit autoreactivity upon bacterial infection and play a role in the pathogenesis of ReA and other spondyloarthropathies. A natural ligand of HLA-B27, spanning residues 169–179 of its own molecule, herein designated as B27-(169–179), was subsequently found in B*2705 and other HLA-B27 subtypes (48, 49). The second region is polymorphic among class I molecules and could provide information about polymorphic HLA-B27 ligands derived from other class I proteins. These peptides would be present by some, but not all, HLA-B27-positive individuals and could be a source of antigenic diversity of HLA-B27 dependent on the non-B27 HLA class I type of the individual.

MATERIALS AND METHODS

Synthetic Peptides—Synthetic peptides were synthesized using standard Fmoc (N-(9-fluorenyl)methoxycarbonyl) chemistry and purified by HPLC. The correct molecular mass of purified peptides was established by matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry (MS), and their quantification was done by amino acid analysis after hydrolysis in 6 M HCl. In cysteine-containing peptides this residue was incorporated as carboxymethyl-Cys during synthesis.

RESULTS

Generation of B27-(169–179) and a C-Terminally Extended 13-mer by the 20 S Proteasome—We first addressed the generation of B27-(169–179), RRYLENGKETL, by the 20 S proteasome from a synthetic 30-mer with the sequence of B*2705 residues 158–187, designated as B27-(158–187). The digestion mixture was fractionated by HPLC (Fig. 1A), and fractions corresponding to absorbance peaks were analyzed by MALDI-TOF and, sometimes, also by quadrupole ion trap nanoelectrospray MS. The yield of individual digestion products was estimated on the basis of their absorbance at 210 nm, normalized to take into account peptic length differences. When various peptides co-eluted, the percentage of each peptide in the absorbance peak was estimated by analyzing for absorbance peaks were analyzed by MALDI-TOF and, sometimes, also by quadrupole ion trap nanoelectrospray MS. The yield of individual digestion products was estimated on the basis of their respective ion peak signal intensities in the MALDI-TOF spectra. This is only an approximation, because ion peak intensity may not strictly correlate with peptide abundance.

About 50% of the B27-(158–187) substrate was digested after 24 h. Of 21 digestion products obtained with >0.1% yield, 13 resulted from cleavage at a single peptide bond, and 8 were internal fragments resulting from dual cleavage (Fig. 1B). Internal fragments accounted for only 5% of the total digestion products. Thus, the relationship between proteasomal cleavage and HLA-B27 ligands must be established not only from the internal fragments observed, but mainly from the analysis of cleaved bonds in the synthetic substrate (Table 1). Cleavage was observed immediately after all four Leu residues: Leu-169, Leu-168, Leu-172, and Leu-175. Cleavages after Leu-168 and Leu-179 are those involved in the generation of the natural B27-(169–179) ligand, although this peptide was not found as a digestion product of B27-(158–187) (Fig. 1C). Cleavage was also observed immediately after any three Arg residues: Arg-169, Arg-170, and Arg-181. The latter peptide bond was the major cleavage point of the B27-(158–187) substrate (Table 1). Dual cleavage after Leu-168 and Arg-181 generated an internal 13-mer, B27-(169–181), with a yield of 0.7% of the total digest (Fig. 1B). This peptide has anchor motifs typical of HLA-B27 ligands (Arg-2, Tyr-3, Arg-13), suggesting that it could exist as...
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In conclusion, proteasomal cleavage in and around the B27-(169–181) sequence was little influenced by its location in the substrate, except near the substrate C terminus. Cleavage at the precise N- and C-terminal ends indicated that B27-(169–181) can be directly produced by the proteasome, as observed with one of the substrates. Cleavage after Arg-181 generated a 13-mer, B27-(169–181), with structural features typical of HLA-B27 ligands.

B27-(169–181) Is a Natural Ligand of B*2705—The B*2705-bound peptide pool was isolated from B*2705-C1R transfectant cells and fractionated by HPLC (Fig. 4A). Fractions collected around the retention time of B27-(169–181) were analyzed by MALDI-TOF MS. An ion peak at mass/charge (m/z) 1662.4, compatible with the molecular mass of the 13-mer, was found in HPLC fraction number 132 (Fig. 4B). The sequence of the corresponding peptide was determined by post-decay MALDI-TOF MS (Fig. 4C) and shown to be B27-(169–181). Thus, this peptide is a natural B*2705 ligand. In the same experiment, B27-(169–179) eluted as the main peptide component of a major absorbance peak (Fig. 4A). On the basis of the absorbance and peptide composition of the corresponding HPLC fraction, the peptide B27-(169–181) was significantly cleaved in the other two precursors, and was observed with much lower yield after Arg-181 (Fig. 3B, Table I). These alterations are presumably explained by the proximity of these bonds to the substrate C terminus, which might impair proteasomal cleavage in its vicinity.

A natural ligand of HLA-B27. Cleavage also occurred after Tyr-171, Asn-174, Thr-178, Gln-180, and Asp-183. Overall, cleavage of B27-(158–187) occurred mainly at two clusters of neighbor peptide bonds: Leu-168-Glu-173 and Thr-178-Pro-184.

These results indicate that the 20S proteasome cleaves B27-(158–187) at the precise peptide bonds required for direct generation of the natural B27-(169–181) ligand. In addition, they suggest the existence of a hitherto unknown B27 ligand. In conclusion, proteasomal cleavage in and around the B27-(169–187) sequence was little influenced by its location in the substrate, except near the substrate C terminus. Cleavage at the precise N- and C-terminal ends indicated that B27-(169–187) can be directly produced by the proteasome, as observed with one of the substrates. Cleavage after Arg-181 generated a 13-mer, B27-(169–181), with structural features typical of HLA-B27 ligands.
peaks B27-(169–179) and B27-(169–181) accounted for about 6 and 0.4% of the total peptide pool, respectively. Thus, the 13-mer was about 15 times less abundant than the 11-mer in the B*2705-bound pool. The abundance of these two ligands is probably due to the high expression of HLA-B27 in the B*2705-C1R transfectant cells. B27-(169–181) is the longest HLA-B27 ligand of known sequence reported so far.

**TABLE I**

Proteasomal cleavage of synthetic substrates mimicking the B*2705 sequence

| Peptide | Amino Acids | Yield (%) |
|---------|-------------|-----------|
| B27-(158–181) yield | B27-(165–194) yield | B27-(154–183) yield | B27-(139–163) yield |
| Ala158 | 74 | 6 |
| Tyr159 | 4 | 1 |
| Leu160 | 0.7 | 5 |
| Gly162 | Not observed | 0.6 |
| Glu166 | Not observed | 0.9 |
| Thr167 | Not observed | Not observed | 1 |
| Leu168 | 0.8 | 9 |
| Arg169 | 0.5 | 2 |
| Arg170 | 5 | 8 |
| Tyr171 | 7 | 8 |
| Leu172 | 2 | 0.8 |
| Asn174 | 0.4 | <0.1 |
| Thr178 | 1 | Not observed |
| Leu179 | 4 | 7 |
| Gln180 | 16 | 3 |
| Arg181 | 48 | 14 |
| Asp183 | 19 | 22 |
| Thr187 | 5 | |
| His188 | 13 | |
| Val189 | 3 | |
| Thr190 | 5 | |
| His191 | 7 | |
| His192 | 5 | |

*a* Only cleavage at bonds in the region overlapping with other substrates in this study is shown. See text for cleavage at other bonds in this substrate.

**FIG. 2**. A, HPLC fractionation of the proteasomal digest of B27-(165–194). About 20 μg of substrate was digested for 24 h at 37 °C with 2 μg of purified proteasome. Peptide products recovered with >0.1% of the total digest (44 of a total of 71) are indicated. Numbering corresponds to the amino acid sequence of HLA-B27. B, digestion pattern of B27-(165–194) by purified 20 S proteasome at 24 h. Conventions are the same as in Fig. 1B.

**FIG. 3**. A, HPLC fractionation of the proteasomal digest of B27-(154–183). About 20 μg of substrate was digested for 24 h at 37 °C with 2 μg of purified proteasome. Peptide products recovered with >0.1% of the total digest (22 of a total of 26), and the undigested 30-mer, are indicated. Numbering corresponds to the amino acid sequence of HLA-B27. B, digestion pattern of B27-(154–183) by purified 20 S proteasome at 24 h. Conventions are the same as in Fig. 1B.
The elution positions of B27-(169–179) and B27-(169–181) were analyzed by MALDI-TOF MS. Alignment of correlating fractions from different subtypes was confirmed by the presence of co-eluting peptides common to different subtypes (Fig. 5). An ion peak at m/z 1662.4, corresponding to B27–169–181, was found in fraction number 132 from B*2705. In this particular chromatography B27–(169–181) co-eluted with many other peptides. The purity of this ligand in its corresponding HPLC fraction, as assessed by MALDI-TOF MS, was variable among different HPLC runs, ranging from being the predominant peptide (Fig. 4B) to eluting in a rather complex peptide mixture (Fig. 5A). The 13-mer was detected only as a very weak signal in the corresponding fraction from B*2709 (Fig. 5A) and was not seen in adjacent HPLC fractions from this subtype (data not shown). Whereas B27–(169–179) was found in B*2709 similarly as in B*2705, that is 4% of the B*2709-bound peptide pool, B27–(169–181) accounted only for 10−%. Thus, the 11-mer/13-mer ratio in B*2709 was about 40,000:1 (Table II).

An ion peak corresponding by molecular mass (m/z: 1662.2) and retention time to B27–(169–181) was also found in B*2704, but not in the corresponding fraction of B*2706 (Fig. 5B) or adjacent ones (data not shown). B27–169–181) was less abundant in B*2704 (0.03%) than in B*2705, but still in the range of many natural class I-bound ligands (62). This lower abundance relative to B*2705 correlates with the lower suitability of the C-terminal Arg residue of this peptide for B*2704 (63, 64). Although in B*2705/B*2709 and B*2704/B*2706 there is a correlation between binding of B27–(169–181) in vivo and subtype association to AS, this correlation was not complete, since the 13-mer was not found in B*2702. B27–(169–179) was abundant in all five subtypes (Table II).

**Generation of Potential HLA-B27 Ligands from a Polymorphic Region of HLA Class I Molecules**—We addressed the possibility that one or more HLA-B27 ligands might arise from proteasomal processing of HLA-B27 or other class I molecules around the polymorphic region spanning residues 150–160. This was suggested by the major cleavage observed after Ala-158 and, to lower extent, Tyr-159, and Leu-160 in B27–154–183 (Fig. 3B), and by the presence of Arg-151, which is conserved among class I HLA molecules. Since HLA-B27 binds peptides with Arg-2, four potential B27 ligands could arise from this region: B27–150–158, ARVAEQLR; B27–150–158, ARVAEQLRA; B27–150–159, ARVAEQLRAY; B27–150–160, ARVAEQLRAY. Thus, a synthetic peptide spanning residues 139–163 of HLA-B*2705, B27–139–163), was digested for 4, 8, and 24 h by the 20 S proteasome, equally as other substrates in this study. Digestion was essentially complete (99%) after 24 h (Fig. 6A). A total of 35 peptides, including 14 external and 21 internal fragments, were obtained with >0.1% yield (Fig. 6B). Cleavage occurred at 17 peptide bonds: after Ala-139 (1%), Ile-142 (8%), Gln-144 (3%), Arg-145 (34%), Trp-147 (4%), Ala-149 (51%), Ala-150 (0.4%), Arg-151 (1%), Val-152 (8%), Ala-153 (5%), Glu-154 (13%), Gln-155 (0.8%), Leu-156 (8%), Arg-157 (0.7%), Ala-158 (6%), Tyr-159 (1%), and Leu-160 (3%). This complexity was not due to the long digestion time, since 80% of the substrate was digested after only 4 h, and the HPLC profile of this digest contained essentially the same peaks as the 24-h digest (data not shown). The peptide bond after Ala-149 was cleaved with the highest efficiency, indicating that the proteasome can generate peptides with the B27 binding motif Arg-2 from this region of the molecule. Cleavage occurred with low yield after Arg-157 and Tyr-159, and better after Ala-158 and Leu-160. Indeed, B27–150–157) was not found in the digest, but B27–(150–158/159/160) were
recovered with 2, 1, and, 0.3% yield, respectively. It is possible that cleavage of some of these bonds is partially impaired by their proximity to the substrate C terminus. In particular, cleavage after Ala-158 was much more efficient in B27-(154–183) (74%) (Table I). Significant cleavage within residues 150–158 (Fig. 6B) also affected the yield of these peptides. Taken together, these results strongly suggest that the proteasome can directly generate four potential B27 ligands, B27-(150–157/158/159/160), and that B27-(150–158) would be the most efficiently produced one.

**B27-(150–158) Is a Natural B*2704 Ligand Arising from a Non-B27 Class I Molecule in the Same Cell**—A search for the B27-(150–157/158/159/160) peptides in the B*2705-bound pool was carried out by MALDI-TOF MS of HPLC fractions around the retention times of these peptides. This screening failed to reveal any major ion peak corresponding to the molecular mass of any of them (data not shown). Using the same approach, B27-(150–158) was found in the B*2704-bound peptide pool from C1R transfectant cells. An ion peak at m/z 1662.2 corresponds to B27-(169–181). Other ion peaks with the same (±1) m/z in both subtypes were labeled.

**TABLE II**

| Subtype | Association to AS | % in the peptide pool |
|---------|-------------------|-----------------------|
|         | B27-(169–179)     | B27-(169–181)         |
| B*2705  | Yes               | 6                     | 0.4                   |
| B*2709  | No                | 4                     | 0.0001                |
| B*2704  | Yes               | 2–0.8                 | 0.03                  |
| B*2706  | No                | 7–4.2                 | Not found             |
| B*2702  | Yes               | 1                     | Not found             |

a See references in the text.

b This was estimated on the basis of the absorbance and peptide composition of the corresponding HPLC peak, relative to the total absorbance of the peptide pool, as described for proteasomal digestion products (see text).

c Data obtained from Edman degradation (49).

the substrate used for in vitro digestion, except at position 163. Therefore it is very likely that B*3503 is the source of the ARVAEQLRA ligand of B*2704. This peptide is an example of a natural HLA-B27 ligand whose expression is dependent on the non-B27 class I molecules present in the cell, and therefore on the HLA type of each individual.

**DISCUSSION**

This study addressed the proteasomal processing of HLA-B27 and other class I molecules, leading to generation of poly-

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**FIG. 5.** A, MALDI-TOF MS spectra corresponding to HPLC fraction numbers 132 (retention time 65 min) of the B*2705-bound (top) and B*2709-bound peptide pool (bottom) from C1R transfectant cells. The ion at m/z 1662.2 corresponds to B27-(169–181). Other ion peaks with the same (±1) m/z in both subtypes are labeled. B, MALDI-TOF MS spectra of HPLC fraction number 132 (retention time 65 min) of the B*2704-bound (top) and B*2706-bound peptide pool (bottom) from C1R transfectant cells. The ion at m/z 1662.2 corresponds to B27-(169–181). Other ion peaks with the same (±1) m/z in both subtypes are labeled.

**FIG. 6.** A, HPLC fractionation of the proteasomal digest of B27-(139–163). About 20 μg of substrate was digested for 24 h at 37 °C with 2 μg of purified proteasome. Peptide products recovered with >0.1% of the total digest (35 of a total of 45), and the undigested 30-mer, are indicated. Numbering corresponds to the amino acid sequence of HLA-B27. B, digestion pattern of B27-(139–163) by purified 20 S proteasome at 24 h. Conventions are the same as in Fig. 1B.
morphic HLA-B27 ligands. First, we analyzed whether the 20 S proteasome cleaved synthetic substrates at the precise N- and C-terminal ends of the natural B27-(169–179) ligand or rather cleavage at the N-terminal end was less precise, favoring generation of N-terminally extended precursors. Second, we assessed internal cleavage within the sequence of the natural ligand and analyzed the influence of substrate structure on cleavage patterns. Third, we applied this analysis to identification of novel HLA-B27 ligands derived from its own or other class I molecules. This approach was recently applied to identification of tumor-associated antigens (65).

Three overlapping synthetic precursors with the sequence of B*2705 in which the B27-(169–179) sequence was placed in N-terminal, central, and C-terminal registers were used to determine cleavage of this region by the 20 S proteasome. Several observations arose from these experiments. First, the 20 S proteasome cleaved efficiently at the precise N- and C-terminal residues of the B27-(169–179) ligand, as also observed for two other HLA-B27 ligands derived from non-HLA proteins (22), and with other MHC class I-bound peptides (8, 20, 24). Second, significant cleavage within the B27-(169–179) sequence may limit the amount of this peptide available for binding to HLA-B27. The cleavage efficiency at peptide bonds leading to generation of this ligand, relative to cleavage of internal bonds might be different in vivo or subjected to differential regulation by PA28. However, the fact that B27-(169–179) was a major component of the B27-bound pool suggests that cleavage of internal bonds does not predominate in vivo over cleavage leading to direct generation of the ligand. Efficient transport into the ER, or its high affinity for HLA-B27 (49), may also influence the abundance of this peptide in the B27-bound pool. Third, proteasomal cleavage after Arg-181 allowed the prediction and identification of the B27-(169–181) 13-mer as a novel HLA-B*2705 ligand containing a sequence homologous to proteins from Gram-negative bacteria (47). Thus, proteasomal cleavage in vitro reproduces, at least in some cases, in vivo processing to the point that it can be used to identify unknown MHC class I ligands.

Significantly, cleavage after Lys-176 was not observed. This would have generated a nonamer, B27-(168–176), or an octamer, B27-(169–176), with B*2705 anchor motifs. The former peptide was initially proposed as the putative peptide mediating molecular mimicry with bacterial peptides in the context of HLA-B27 (47), but it has not been found as a natural B27 ligand. The cleavage pattern observed in this region explains this absence and suggests that the presence of these two peptides in the B27-bound pool is unlikely.

Polymorphism at flanking positions may substantially alter proteasomal cleavage in and around a given peptide sequence (15–19). An important aspect of in vitro digestions that has seldom been specifically addressed is to what extent the location of a particular sequence within the precursor substrate may influence cleavage patterns. Our results demonstrated that proteasomal specificity around the sequence of a given ligand was little influenced by its precise location within the substrate. The main difference was observed in peptide bonds close to the C-terminal end of some substrates. For instance, impaired cleavage at residues 179–181 in B27-(154–183) relative to B27-(158–187), and at residues 158–160 in B27-(139–163) relative to B27-(154–183), is presumably due to the proximity of the negatively charged C terminus (9).

The pathogenetic significance of presentation by HLA-B27 of
two peptides derived from its own molecule containing a sequence with homology to proteins from Gram-negative bacteria is unclear. That B27-(169–179) is a prominent natural ligand of HLA-B27 subtypes associated and not associated to AS suggests that it is not relevant to this disease. However, the possibility that it may be arthritogenic only in the context of some subtypes cannot be ruled out. In addition, autoreactive CD8+ cytolytic T lymphocytes with specificity for B27-(168–176) have been detected in AS patients. As mentioned above, there is no evidence for this peptide being a natural B27 ligand, nor is it produced by the 20S proteasome in vitro, the finding raises the possibility that natural HLA-B27 ligands containing this sequence may play a role in disease. If so, the finding of B27-(169–181) might have some significance. This peptide has a C-terminal Arg residue, which is disfavored for binding to B+2706 and B+2709, subtypes not associated with AS. Indeed, this 13-mer was not found in B+2706 and was in very low amount in B+2709, whereas it was prominent in the disease-associated B+2705 and B+2704. However, correlation of this peptide with association to AS, although better than for B27-(169–179), was not complete, since it was apparently absent from the disease-associated B+2702 subtype.

The region spanning residues 150–158 includes three positions that are polymorphic among class I molecules: 152, 156, and 158 (66). Of these, position 152 is either Val or Glu, both among class I proteins and HLA-B27 subtypes (67). That B27-(150–158) was directly generated from a synthetic precursor and found as a B+2704 ligand in C1R transfectant cells again shows that proteasome cleavage in vitro is suitable for predicting novel class I MHC ligands. As noted above, this peptide probably arose from B+3503, expressed at reduced levels in C1R cells (50). Thus, it is a polymorphic HLA-B27 ligand whose expression is genetically determined and dependent on the comitant presence of certain non-B27 HLA class I molecules. Peptides such as this one introduce diversity among B27-bound peptide repertoires from different individuals.

Proteasomal degradation of misfolded class I polypeptides after dislocation from the ER to the cytosol is probably a physiological quality control process (68), but becomes especially relevant in situations that favor class I misfolding (46). These might be, for instance, intracellular bacterial infections or stimulation of class I protein synthesis during inflammation. If polymorphic peptides derived from other class I molecules and presented by HLA-B27 would play a pathogenic role in HLA-B27-associated disease, this could contribute to explain that only a fraction of the B27-positive individuals develop spondyloarthopathies. If potentially pathogenic peptide sequences were encoded by few class I HLA alleles, these would probably show up as additional genetic markers for these diseases. However, if such peptide sequences were encoded by multiple non-B27 class I alleles, their association with spondyloarthopathy would be much more difficult to detect by conventional genetic analysis. Indeed, besides a significant contribution of non-MHC genes (>50%), an additional contribution of non-B27 genes within the MHC to AS is supported by genetic studies (45).

In conclusion, three natural HLA-B27 ligands, derived from the B27 molecule itself or other class I proteins, were directly generated by the 20S proteasome in vitro. Although protein processing in vivo may be different to some extent due to substrate differences and to involvement of PA28, cleavage of synthetic precursors in vitro explains the presence of the natural ligands analyzed in this study. The correspondence between in vitro cleavage patterns and generation of natural ligands was illustrated by prediction and finding of novel class I-derived HLA-B27 ligands.


due to the page limitation, the full references are not included here. The full text is available in the cited references or through the provided links.
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Identification of Novel HLA-B27 Ligands Derived from Polymorphic Regions of Its Own or Other Class I Molecules Based on Direct Generation by 20 S Proteasome

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