OVERLAPPING PEPTIDE-BINDING SPECIFICITIES OF HLA–B27 AND B39

Evidence for a Role of Peptide Supermotif in the Pathogenesis of Spondylarthropathies

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Objective. Previous studies indicated the increase of HLA–B39 among HLA–B27 negative patients with spondylarthropathies (SpA). This study was performed to examine whether the natural ligands of HLA–B27 are capable of binding to HLA–B39.

Methods. Peptides were synthesized according to the sequences of known natural ligands of HLA–B27 or B39 and were tested for their binding to HLA–B*3901 and B*2705 by quantitative peptide binding assay, using a TAP-deficient RMA-S cell line transfected with human β2-microglobulin and HLA class I heavy chain genes.

Results. Four of the 10 HLA–B27 binding peptides significantly bound to HLA–B*3901. All 4 peptides had hydrophobic/aromatic amino acids (Leu or Phe) at the C-terminus. In contrast, peptides with basic residues (Lys, Arg) or Tyr at the C-terminus did not bind to B*3901. In parallel experiments, 1 of the 2 natural ligands of HLA–B*3901 was found to bind to B*2705.

Conclusion. A subset of natural HLA–B27 ligands was capable of binding to B*3901. In addition to Arg at position 2 (Arg2), hydrophobic/aromatic C-terminal residues, such as Leu or Phe, seemed to be crucial for the cross-specificity. These results suggested that HLA–B27 and B39 recognize overlapping peptide repertoires, supporting the hypothesis that the peptides presented by both of these class I antigens play a role in the pathogenesis of SpA.

Although the crucial role of HLA–B27 in the development of seronegative spondylarthropathies (SpA) has been validated in transgenic animals (1,2) and in human subjects through linkage studies (3), how HLA–B27 causes the disease remains an open question. Hypotheses such as molecular mimicry, presentation of arthritogenic peptides, influence on bacterial invasion or persistence, and B27-mediated modification of intracellular signaling are currently being tested (4).

The molecular feature that most strikingly distinguishes HLA–B27 from other class I antigens lies in the structure of the peptide-binding pocket B (5). This structure is related to the peptide-binding motif of HLA–B27 possessing Arg at position 2 (Arg2), which is rather unique among class I molecules (6). We previously reported an increase of HLA–B39 in HLA–B27 negative Japanese patients with SpA (7). Of interest, HLA–B27 and HLA–B*3901, the predominant subtype of B39, share some of the polymorphic amino acid residues constituting pocket B (Glu45, Cys67). In fact, the peptide motif of HLA–B*3901 has also been shown to have Arg2 as well as His2 (8). These observations led us to postulate that a subset of the peptides presented by HLA–B27 could also be presented by HLA–B39, and that the peptides of pathogenic significance might be present in such a group of peptides.

This study was performed to experimentally evaluate the hypothesis that a proportion of peptides bound by HLA–B27 is capable of binding to HLA–B*3901.

MATERIALS AND METHODS

Peptides. Peptides were synthesized utilizing an automated multiple peptide synthesizer utilizing the Fmoc strategy.
followed by cleavage, as previously described (9). Peptides were shown to be homogeneous by reverse-phase high-pressure liquid chromatography and mass spectrometry. Peptides 2702-1 to 2702-4 and 2705-1 to 2705-6 were randomly selected from a panel of natural ligands of B*2702 and B*2705, respectively (10,11). Peptides 3901-1 and 3901-2 were also derived from the previously identified natural ligands of B*3901 (8). An irrelevant peptide without B27 or B39 binding motif, LFKDWEEL, was included as a negative control.

**Cells.** RMA-S is a mutant cell line derived from mouse T cell lymphoma, expressing class I major histocompatibility complex molecules that form unstable heterodimers with β2-microglobulin (β2m) because of the lack of TAP genes. RMA-S cells expressing transfected human class I antigens and human β2m form class I molecule–peptide complexes (stable at 37°C) only when peptides capable of binding to the class I molecules are supplied exogenously (9).

RMA-S transfectants expressing human β2m and HLA–B*2705 (RMA-S–B*2705) and B*3901 (RMA-S–B*3901) were established as previously described (9). RMA-S cells expressing human β2m (RMA-S–hβ2m) were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS). The genomic HLA–B*2705 gene, generously provided by Dr. J. A. López de Castro (Universidad Autónoma de Madrid, Madrid, Spain), or genomic B*3901 gene (12) was transfected by electroporation with a hygromycin-resistance gene into RMA-S–hβ2m cells. After selection by hygromycin B (0.75 mg/ml), hygromycin-resistant cells were isolated from separate wells. Surface expression of HLA class I antigens and human β2m was detected by flow cytometry with a monoclonal antibody (mAb) to HLA class I α3 domain, TP25.99 (13), after culture for 3 hours at 26°C. RMA-S–B*2705 and RMA-S–B*3901 were maintained in RPMI 1640 medium containing 10% FCS and 0.2 mg/ml of hygromycin B.

In addition, RMA-S cells expressing B*3501 (RMA-S–B*3501) (9), B*5101 (RMA-S–B*5101) (14,15), and B*5301 (RMA-S–B*5301) (Takiguchi M et al: manuscript in preparation) were used as control transfectants to test the specificity of the binding assay.

**Peptide binding assay.** The binding of peptides to HLA–B*2705 and B*3901 molecules was tested using flow cytometry as previously described (14,15). RMA-S–B*2705 and RMA-S–B*3901 cells were cultured for 18–24 hours at 26°C. Cells (2 × 10^5) in 50 μl of phosphate buffered saline (PBS) supplemented with 20% FCS (PBS-FCS) were incubated at 26°C for 3 hours with 50 μl of a solution of peptides at 1 × 10^-7, 1 × 10^-6, 3.3 × 10^-6, 1 × 10^-5, 3.3 × 10^-5, 1 × 10^-4, 3.3 × 10^-4, and 1 × 10^-3 molar and subsequently at 37°C for 3 hours. After washing with PBS-FCS, cells were incubated for 30 minutes on ice with an appropriate dilution of fluorescein isothiocyanate–conjugated sheep anti-mouse immunoglobulin (Silenus Laboratories, Hawthorn, Australia). Cells were then washed 3 times, and fluorescence intensity was measured using FACScan.

The mean fluorescence intensity (MFI) of RMA-S cells expressing introduced B*2705 or B*3901 cultured at 26°C without peptides was considered maximal binding, whereas the MFI of cells cultured at 26°C for 3 hours and subsequently at 37°C for 3 hours without peptides was considered the background. Peptides giving >25% of the MFI of maximal binding at a concentration of 10^-3 M after subtraction of the background were considered binding peptides. The peptide concentration yielding half-maximal binding was calculated as described elsewhere (15).

**RESULTS**

**Generation of RMA-S–hβ2m cells expressing HLA–B*2705 or B*3901.** RMA-S–hβ2m cells expressing HLA–B*2705 or B*3901 were generated by the trans-
fection of genomic DNA encoding HLA–B*2705 or B*3901, respectively, into RMA-S–hβ2m cells. The surface expression of HLA molecules on these transfectants was examined using TP25.99 anti–HLA class I α3 domain after the cells were cultured overnight at 26°C or at 37°C.

As shown in Figure 1, surface expression of HLA–B*2705 and B*3901 was induced after the cells were cultured at 26°C and diminished after the cells were subsequently cultured at 37°C for 3 hours. These results indicated that both cell lines can be used for the peptide binding assay.

Binding of a panel of natural ligands of B*2705 or B*2702 molecules to the transfectants. To examine whether a subset of peptides naturally bound by HLA–B27 is also capable of binding to HLA–B39, 10 peptides were randomly selected from a panel of natural ligands of HLA–B*2705 (peptides 2705-1 to 2705-6) or B*2702 (peptides 2702-1 to 2702-4), as shown in previous reports (10,11), and were tested for binding to RMA-S–B*2705 and to RMA-S–B*3901. More than 25% of the maxi-
mal binding at a peptide concentration of $10^{-3}M$ was considered significant. As expected, all peptides showed significant binding to RMA-S–B*2705 (Figures 2A and B).

The binding of the peptides to RMA-S–B*3901 is shown in Figure 3. Three of 6 natural ligands of B*2705 (2705-1, 2, and 3), as well as 1 of 4 natural ligands of B*2702 (2702-2), demonstrated significant binding to RMA-S–B*3901.

**Binding of natural ligands of the B*3901 molecule to the transfectants.** In parallel experiments, 2 natural ligands of B*3901 were examined for binding to the 2 cell lines. Both peptides showed strong binding to RMA-S–B*3901 (Figure 4A). In addition, 1 of the peptides (3901-1) demonstrated significant binding to RMA-S–B*2705 (Figure 4B).

**Association of peptide motifs with the cross-specificity.** The peptide binding assays were repeated 3 times, and the results were similar. The affinity of binding of the tested peptides to the transfectants is summarized in Table 1. It was noted that B27-binding peptides with cross-specificity to B*3901 (2705-1, 2705-2, 2705-3, and 2702-1) characteristically possessed hydrophobic/aromatic acids (Phe, Leu) at the C-terminus. Of 5 such peptides, 4 demonstrated significant binding to B*3901. On the other hand, peptides that possess either basic (Lys, Arg; 2705-4, 2705-5, 2705-6) or polar (Tyr; 2702-3, 2702-4) amino acids at the C-terminus did not exhibit significant binding to B*3901.

Among the B39-binding peptides, the one that showed cross-specificity to B*2705 possessed Arg at position 2 (3901-1), while the other peptide, with His at position 2, did not bind to B*2705.

**Binding of the peptides to control class I transfectants.** To confirm the specificity of the binding assay, the natural ligands of B27 and B39 were tested for binding to RMA-S cells transfected with B*3501, B*5101, or B*5301. Among the 10 ligands of B27 and 2 ligands of B39, no peptide showed binding to RMA-S–B*5101 or RMA-S–B*5301, and only 1 (2702-1) showed weak binding to RMA-S–B*3501 (results not shown). These results indicated that the cross-specificity between B27 and B39 cannot be explained by an experimental artifact.

**DISCUSSION**

In the present study, we demonstrated that a subset of natural ligands of HLA–B27 is capable of binding to HLA–B*3901. These results and our previous observations showing the association of HLA–B39 with SpA among HLA–B27 negative Japanese individuals (7) support the hypothesis that the peptides presented by HLA–B27 and B39 in common may play a significant role in the pathogenesis of SpA.

The peptide motif for HLA–B27 and its subtypes have been extensively studied. Most of the studies demonstrated that position 2 and the C-terminal residues (usually position 9) constitute the primary anchor residues (16). The position 2 residues of most of the peptides eluted from HLA–B*2705, B*2702, or B*2703 are Arg (10,11,17). Peptide binding assays using synthetic peptides showed the dominance of Arg in all B27 subtypes studied (18–20), although Val, Gln, and His...
Table 1. Sequences of the tested peptides and their affinity to RMA-S–B*2705 and RMA-S–B*3901

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Sequence</th>
<th>% binding†</th>
<th>BL_{50} (M)</th>
<th>% binding†</th>
<th>BL_{50} (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2705-1</td>
<td>RRFGDKLFN</td>
<td>119.4‡</td>
<td>6.9 \times 10^{-5}</td>
<td>50.1‡</td>
<td>1 \times 10^{-5}</td>
</tr>
<tr>
<td>2705-2</td>
<td>RRYQKSTEL</td>
<td>118.4‡</td>
<td>3 \times 10^{-5}</td>
<td>107.8‡</td>
<td>1.9 \times 10^{-5}</td>
</tr>
<tr>
<td>2705-3</td>
<td>ARLQTALL</td>
<td>44.2‡</td>
<td>&gt;10^{-3}</td>
<td>50.0‡</td>
<td>1 \times 10^{-3}</td>
</tr>
<tr>
<td>2705-4</td>
<td>GRIDKPILK</td>
<td>125.8‡</td>
<td>3.1 \times 10^{-5}</td>
<td>-3.1</td>
<td>-</td>
</tr>
<tr>
<td>2705-5</td>
<td>ARLFGIRAK</td>
<td>108.1‡</td>
<td>1.1 \times 10^{-4}</td>
<td>10.7</td>
<td>-</td>
</tr>
<tr>
<td>2705-6</td>
<td>RRSKEITVR</td>
<td>107.6‡</td>
<td>9.2 \times 10^{-6}</td>
<td>-1.1</td>
<td>-</td>
</tr>
<tr>
<td>2702-1</td>
<td>RRFNVVPTFT</td>
<td>109.7‡</td>
<td>1.3 \times 10^{-5}</td>
<td>40.4‡</td>
<td>&gt;10^{-3}</td>
</tr>
<tr>
<td>2702-2</td>
<td>GRLTKHTKF</td>
<td>115.1‡</td>
<td>4 \times 10^{-5}</td>
<td>2.4</td>
<td>-</td>
</tr>
<tr>
<td>2702-3</td>
<td>KRYSIVKVO</td>
<td>114.8‡</td>
<td>1.4 \times 10^{-5}</td>
<td>-8.0</td>
<td>-</td>
</tr>
<tr>
<td>2702-4</td>
<td>KRGGITLTKY</td>
<td>111.2‡</td>
<td>4.2 \times 10^{-5}</td>
<td>-9.8</td>
<td>-</td>
</tr>
<tr>
<td>3901-1</td>
<td>SRIKDIIM</td>
<td>83.2‡</td>
<td>1.1 \times 10^{-3}</td>
<td>97.3‡</td>
<td>9.6 \times 10^{-5}</td>
</tr>
<tr>
<td>3901-2</td>
<td>SHIGDAVV</td>
<td>-16.0</td>
<td>-</td>
<td>87.4‡</td>
<td>1.8 \times 10^{-4}</td>
</tr>
<tr>
<td>Control</td>
<td>LFKDWEEL</td>
<td>-17.1</td>
<td>-</td>
<td>-2.6</td>
<td>-</td>
</tr>
</tbody>
</table>

* The amino acid sequences of the peptides were derived from ref. 8 (3901-1 and 2), ref. 10 (2705-1, 3, and 5; 2702-1, 2, 3, and 4), and ref. 11 (2705-2, 4, 5, and 6). BL_{50} = half-maximal binding level.
† % binding = 
\[
\frac{\text{MFI at the peptide concentration of } 10^{-5} \text{M} - \text{background MFI}}{\text{maximal MFI} - \text{background MFI}} \times 100
\]
‡ Peptides showing >25% binding were considered to be binding peptides.

Also seem to be accepted (19,21). On the other hand, substantial variations have been shown for the position 9 anchor among B27 subtypes. While peptides with basic amino acids (Lys, Arg, His) and hydrophobic or aromatic amino acids (Leu, Phe, Tyr, Ile, Met) at position 9 can be bound by HLA–B*2705, subtypes such as B*2702, B*2704, B*2706, B*2707, or B*2709 seem to bind hydrophobic/aromatic amino acids preferentially (10,20,22,23). In addition, peptides with C-terminal Tyr bind hydrophobic/aromatic amino acids preferentially to B*2706 and B*2709 (22,24). Of interest, 4 studies have been shown to bind to B*2702 and B*2704, but not B*2706, B*2707, or B*2709 (29). On the other hand, significant association with HLA–B39 was reported to be associated with reactive arthritis induced by tonsillitis (33,34). On the other hand, significant association with HLA–B39 was not observed in the United Kingdom, although a slight tendency toward increase seems to be present (35). Similarly, in Lebanon, where the proportion of HLA–B27 positive patients with SpA is unusually small, a slight, but not significant, increase of HLA–B38, which possesses a peptide motif similar to that of B39 (8), was reported in SpA patients (36). Although the reason for such inconsistency among populations is unclear, differences in the triggering environmental factors, genetic background other than HLA–B27, or the phenotype of the diseases among the populations might be involved. The small risk conferred by HLA–B39 is probably explained by the lower affinity
of the disease-associated peptides for HLA–B39 compared with the affinity for HLA–B27.

Several studies indicated the association of HLA–B40 with SpA (3,37,38). Although it would be interesting to test the binding of B27 ligands to B40 in future studies, it seems to be unlikely that B27 and B40 possess overlapping peptide specificities, because the reported B40 peptide motif includes Glu at position 2 (39). It is possible that B40 can present a different set of arthritogenic peptides other than those presented by B27. Mechanisms other than peptide presentation also cannot be excluded.

Our present study suggested that HLA–B27 and B39 share the supermotif of natural peptide ligands (40), and that the peptides of pathologic significance should be compatible with such a supermotif. Peptide binding experiments using synthetic peptide analogs with substitutions of the C-terminal residues of each of the hydrophobic/aromatic amino acids will be useful to further narrow the pathogenic peptide motifs. Identification of endogenous peptides bearing such a supermotif, which are preferentially expressed in cells constituting the axial joints, will eventually help in our understanding of the role of HLA–B27 in the development of SpA.

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REFERENCES

toire from those of B*2704 and other HLA-B27 subtypes associ-
25. López-Larrea C, Su jirachato K, Mehra NK, Chiewsli P,
Isarangkura D, Kanga U, et al. HLA-B27 subtypes in Asian
patients with ankylosing spondylitis: evidence for new associations.
26. Ren EC, Koh WH, Sim D, Boey ML, Wee GB, Chan SH. Possible
protective role of HLA-B*2706 or ankylosing spondylitis. Tissue
27. Nasution AR, Mardjuadi A, Kunmartini S, Suryadhana NG,
Setyohadi B, Sudarsono D, et al. HLA-B27 subtypes positively and
28. Gonzalez-Roces S, Alvarez MV, Gonzalez S, Dieye A, Makni H,
Woodfield DG, et al. HLA-B27 polymorphism and worldwide
susceptibility to ankylosing spondylitis. Tissue Antigens 1997;49:
116–23.
29. D'Amato M, Fiorillo MT, Carcassi C, Mathieu A, Zuccarelli A,
Bitti PP, et al. Relevance of residue 116 of HLA-B27 in determin-
ing susceptibility to ankylosing spondylitis. Eur J Immunol 1995;
25:3199–201.
30. Khan MA, Kushner I, Braun WE. B27-negative HLA-Bw16 in
31. Hall PJ, Burman SJ, Laurent MR, Briggs DC, Venning HE, Leak
AM, et al. Genetic susceptibility to early onset pauciarticular
juvenile chronic arthritis: a study of HLA and complement mark-
32. Salvarani C, Macchioni P, Cremonesi T, Mantovani W, Battistel B,
Rossi F, et al. The cervical spine in patients with psoriatic arthritis:
a clinical, radiological and immunogenetic study. Ann Rheum Dis
Reactive arthritis induced by tonsillitis [abstract]. Arthritis Rheum
1992;35 Suppl 9:S244.
Reactive arthritis induced by Pseudomonas aeruginosa. Clin
35. Brown M, Bunce M, Calin A, Darke C, Wordsworth P. HLA-B
associations of HLA-B27 negative ankylosing spondylitis: comment
on the article by Yamaguchi et al [letter]. Arthritis Rheum
R, et al. Weak association between HLA-B27 and the spondyloar-
37. Robinson WP, van der Linden SM, Khan MA, Rentsch H-U, Cats
A, Russell A, et al. HLA-Bw60 increases susceptibility to ankylos-
ing spondylitis in HLA–B27+ patients. Arthritis Rheum 1989;32:
1135–41.
38. Brown MA, Kennedy LG, MacGregor AJ, Drake C, Duncan E,
Shatford JL, et al. Susceptibility to ankylosing spondylitis in twins:
the role of genes, HLA, and the environment. Arthritis Rheum
et al. Peptide motifs of HLA-B58, B60, B61 and B62 molecules.
40. Sidney J, Grey HM, Kubo RT, Sette A. Practical, biochemical and
evolutionary implications of the discovery of HLA class I super-

Erratum

In the Reply letter by Cid et al published in the November 1998 issue of Arthritis & Rheumatism (pp 2088–2089), the name of the eighth author was spelled incorrectly. The correct spelling of the author’s name is Joaquim Oristrell. We regret the error.