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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 β_2-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 (Arg^2), 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.**

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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 (Arg^2), 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 (Glu^{45}, Cys^{67}). In fact, the peptide motif of HLA–B*3901 has also been shown to have Arg^2 as well as His^2 (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 β_2-microglobulin (β_2m) because of the lack of TAP genes. RMA-S cells expressing transfected human class I antigens and human β_2m 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 β_2m 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 β_2m (RMA-S–hβ_2m) 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–hβ_2m 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 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 TP25.99 mAb. After two washes 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 fluorescein 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).

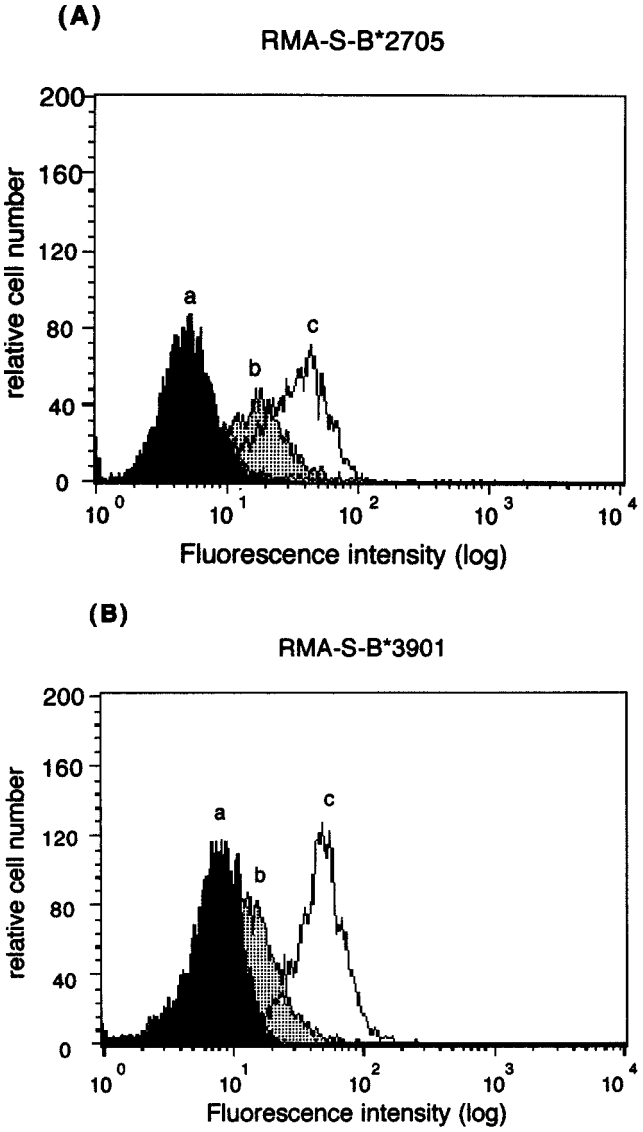

Figure 1. Surface expression of HLA–B*2705 **(A)** or HLA–B*3901 **(B)** molecules on RMA-S cells expressing human β_2-microglobulin (RMA-S–hβ_2m) transfected with the HLA class I heavy chain genes. Cells were cultured at 37°C **(a),** at 26°C **(c),** and at 26°C for 3 hours and then at 37°C for 3 hours without exogenous peptides **(b)**.

RESULTS

Generation of RMA-S–hβ_2m cells expressing HLA–B*2705 or B*3901. RMA-S–hβ_2m cells expressing HLA–B*2705 or B*3901 were generated by the trans-

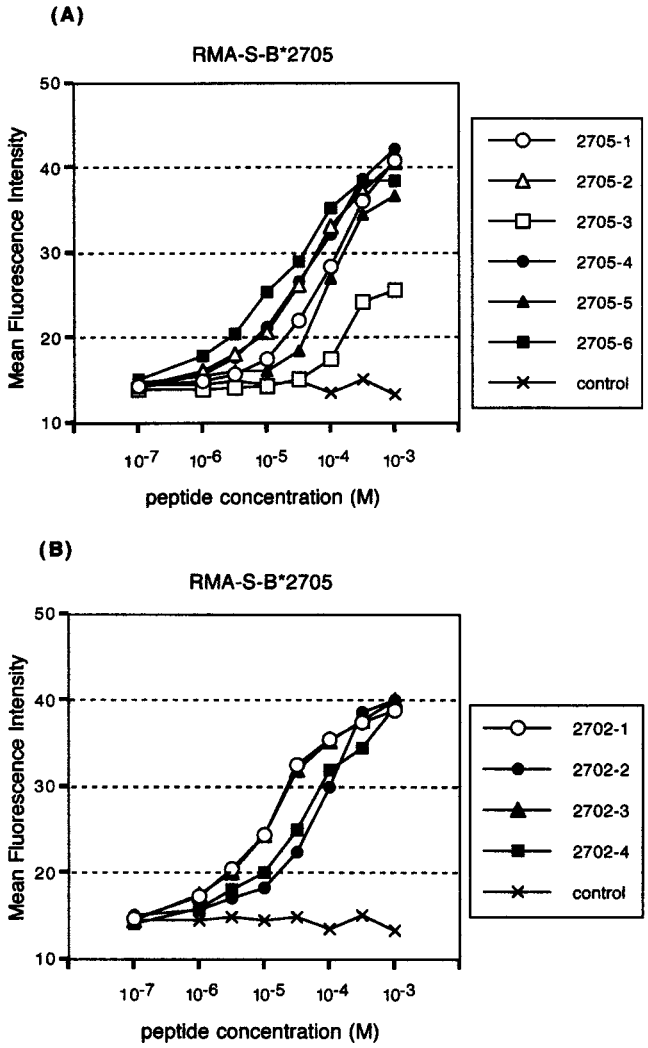

Figure 2. Binding of natural ligands of B*2705 (**A**) and B*2702 (**B**) to RMA-S–B*2705 cells. RMA-S–B*2705 cells were loaded with the peptides at 26°C, and then incubated for 3 hours at 37°C. Cells were stained by indirect immunofluorescence using monoclonal antibody to HLA class I α3 domain, TP25.99. All peptides except the control showed significant binding to B*2705. The mean fluorescence intensity of RMA-S–B*2705 cells cultured at 26°C was 37.03.

fection of genomic DNA encoding HLA–B*2705 or B*3901, respectively, into RMA-S–hβ_2m 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-

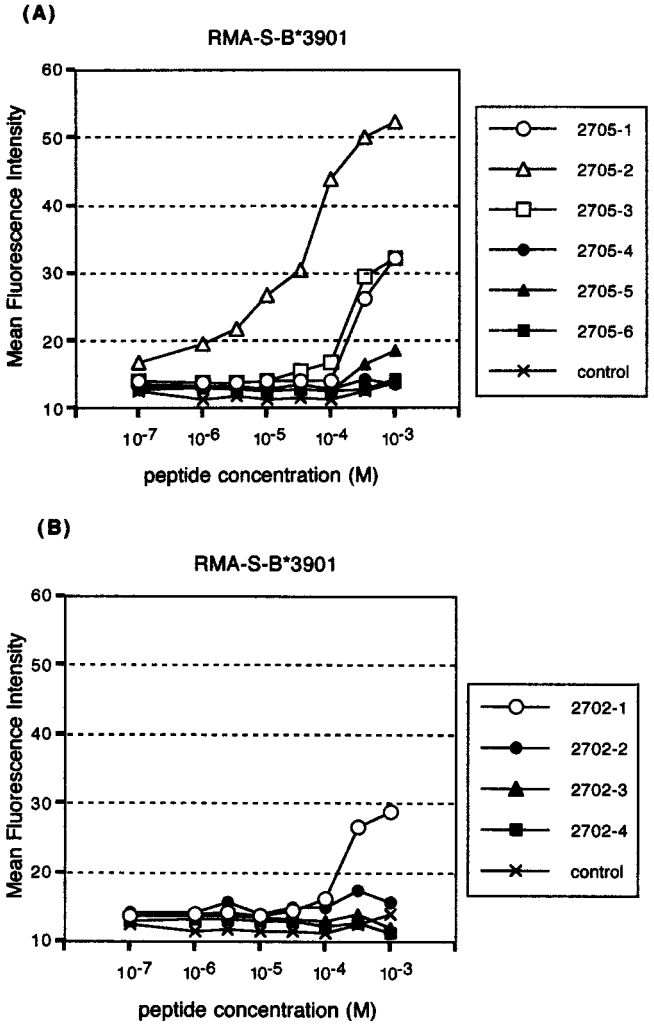

Figure 3. Binding of natural ligands of B*2705 (**A**) and B*2702 (**B**) to RMA-S–B*3901 cells. Assays were performed as described in Figure 2. Among 6 natural ligands of B*2705, 2705-1, 2705-2, and 2705-3 showed significant binding to B*3901 (**A**). Among 4 natural ligands of B*2702, 2702-1 significantly bound to B*3901 (**B**). The mean fluorescence intensity of RMA-S–B*3901 cells cultured at 26°C was 49.63. The assay was repeated 3 times, with similar results.

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.

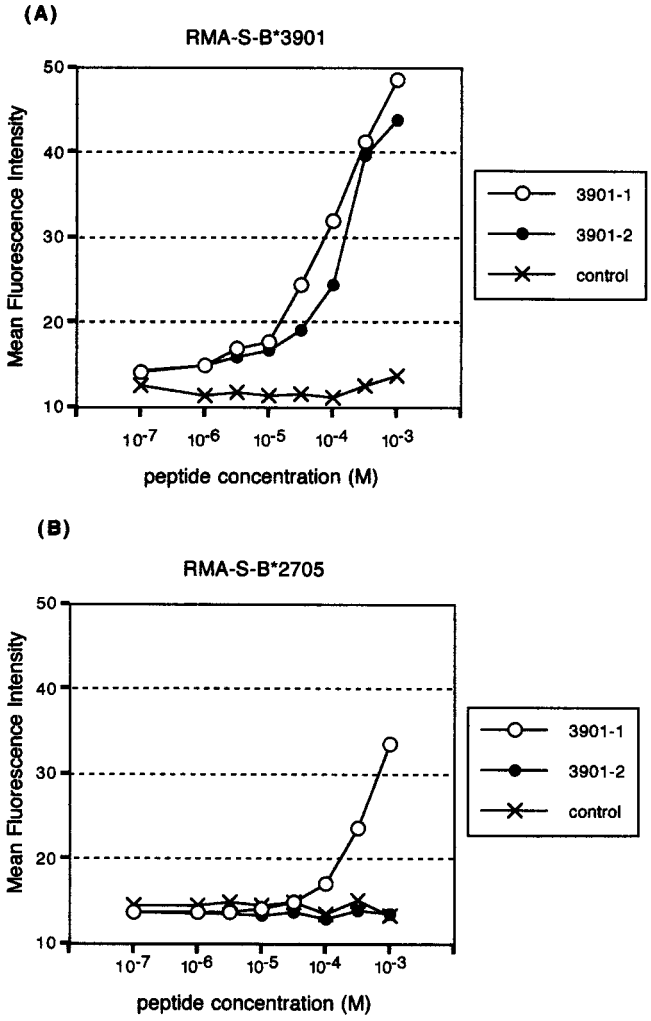

Figure 4. Binding of natural ligands of B*3901 to B*3901 **(A)** and B*2705 **(B)**. Assays were performed as described in Figure 2. Both peptides strongly bound to B*3901, as expected **(A)**. In addition, 3901-1 showed significant binding to B*2705 **(B)**. The assay was repeated 3 times, with similar results.

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[2] 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*

		RMA-S–B*2705		RMA-S–B*3901	
Peptide	Sequence	% binding†	BL_{50} (M)	% binding†	BL_{50} (M)
2705-1	RRFGDKLNF	119.4‡	6.9×10^{-5}	50.1‡	1×10^{-3}
2705-2	RRYQKSTEL	118.4‡	3×10^{-5}	107.8‡	1.9×10^{-5}
2705-3	ARLQTALL	44.2‡	$>10^{-3}$	50.0‡	1×10^{-3}
2705-4	GRIDKPILK	125.8‡	3.1×10^{-5}	−3.1	–
2705-5	ARLFGIRAK	108.1‡	1.1×10^{-4}	10.7	–
2705-6	RRSKEITVR	107.6‡	9.2×10^{-6}	−1.1	–
2702-1	RRFVNVVPTF	109.7‡	1.3×10^{-5}	40.4‡	$>10^{-3}$
2702-2	GRLTKHTKF	115.1‡	4×10^{-5}	2.4	–
2702-3	KRYKSIVKY	114.8‡	1.4×10^{-5}	−8.0	–
2702-4	KRGILTLKY	111.2‡	4.2×10^{-5}	−9.8	–
3901-1	SRDKTIIM	83.2‡	1.1×10^{-3}	97.3‡	9.6×10^{-5}
3901-2	SHIGDAVV	−16.0	–	87.4‡	1.8×10^{-4}
Control	LFKDWEEL	−17.1	–	−2.6	–

* 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 = $\dfrac{\text{MFI at the peptide concentration of } 10^{-3}M - \text{background MFI}}{\text{maximal MFI} - \text{background MFI}} \times 100$

‡ 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 have been shown to bind to B*2702 and B*2704, but not to B*2706 and B*2709 (22,24). Of interest, 4 studies carried out in Asian populations convincingly demonstrated a very low association between B*2706 and ankylosing spondylitis (AS) (25–28). Moreover, a study in Sardinia showed the lack of association between B*2709 and AS (29).

Based on these observations, it has been proposed that the potential disease-associated antigenic peptides should possess Tyr at the C-terminus (22,24). This hypothesis, however, was not supported by the recent report by Tieng and associates, which showed that the peptides naturally presented by B*2707 do not have Tyr at the C-terminus, although B*2707 is associated with SpA (23).

Our present study indicated that, among the natural ligands of B*2705 and B*2702, those possessing Phe or Leu at the C-terminus can bind to B*3901, while peptides with basic amino acids or Tyr at the C-terminus cannot bind to B*3901. Based on these observations, it is proposed that the potential arthritogenic peptides should possess Arg at position 2 and hydrophobic/aromatic amino acids other than Tyr—most likely Phe or Leu—at the C-terminus. The lack of disease association with B*2706 and B*2709 may possibly be related to the difference in the preference of minor anchor residues, rather than the C-terminal residue. This interpretation is consistent with the report by Tieng et al (23). Although the natural ligands of B*3901 exhibited preference for hydrophobic amino acids at position 6 (8), such preference did not seem to be critical for binding when the tested peptides had the motif described above.

The preference of the position 9 residues are considered to be substantially affected by the structure of the F pocket (25). For example, the negative charge provided by Asp^{77} in B*2705 is associated with the preference of positively charged amino acids at position 9, while the substitution of Tyr (B*2706, B*2707) or His (B*2709) for Asp^{116} seems to result in the incompatibility of Tyr as the C-terminal anchor (23,24,29). The structure of the pocket F of B*3901 is identical with B*2708, except for the substitution of Phe for Asp at position 116. It is likely that the steric hindrance by the aromatic group of Phe^{116} would result in the exclusion of Tyr at position 9 from the B*3901 peptide motif.

The association of HLA–Bw16 (B38, B39) with HLA–B27 negative patients with AS was originally reported in Caucasians (30). The association of HLA–B39 with early-onset pauciarticular juvenile rheumatoid arthritis (31) and with spondylitis in patients with psoriatic arthritis (32) was also reported in Caucasians. In Japanese, in addition to our observations, 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

1. Hammer RE, Maika SD, Richardson JA, Tang JP, Taurog JD. Spontaneous inflammatory disease in transgenic rats expressing HLA-B27 and human β2m: an animal model of HLA-B27-associated human disorders. Cell 1990;63:1099–112.
2. Khare SD, Luthra HS, David CS. Spontaneous inflammatory arthritis in HLA-B27 transgenic mice lacking β_2-microglobulin: a model of human spondyloarthropathies. J Exp Med 1995;182: 1153–8.
3. Rubin LA, Amos CI, Wade JA, Martin JR, Bale SJ, Little AH, et al. Investigating the genetic basis for ankylosing spondylitis: linkage studies with the major histocompatibility complex region. Arthritis Rheum 1994;37:1212–20.
4. Feltkamp TEW, Khan MA, López de Castro JA. The pathogenetic role of HLA-B27. Immunol Today 1996;17:5–7.
5. Madden DR, Gorga JC, Strominger JL, Wiley DC. The three-dimensional structure of HLA-B27 at 2.1 Å resolution suggests a general mechanism for tight peptide binding to MHC. Cell 1992;70:1035–48.
6. Davenport MP, Hill AVM. Peptides associated with MHC class I and class II molecules. In: Browning MJ, McMichael AJ, editors. HLA and MHC: genes, molecules and function. Oxford: BIOS Scientific Publishers Ltd; 1996. p. 277–308.
7. Yamaguchi A, Tsuchiya N, Mitsui H, Shiota M, Ogawa A, Tokunaga K, et al. Association of HLA-B39 with HLA-B27-negative ankylosing spondylitis and pauciarticular juvenile rheumatoid arthritis in Japanese patients: evidence for a role of the peptide-anchoring B pocket. Arthritis Rheum 1995;38:1672–7.
8. Falk K, Rötzschke O, Takiguchi M, Gnau V, Stevanović S, Jung G, et al. Peptide motifs of HLA-B38 and B39 molecules. Immunogenetics 1995;41:162–4.
9. Takamiya Y, Schönbach C, Nokihara K, Yamaguchi M, Ferrone S, Kano K, et al. HLA-B*3501-peptide interactions: role of anchor residues of peptides in their binding to HLA-B*3501 molecules. Int Immunol 1994;6:255–61.
10. Rötzschke O, Falk K, Stevanović S, Gnau V, Jung G, Rammensee H-G. Dominant aromatic/aliphatic C-terminal anchor in HLA-B*2702 and B*2705 peptide motifs. Immunogenetics 1994;39: 74–7.
11. Jardetzky TS, Lane WS, Robinson RA, Madden DR, Wiley DC. Identification of self peptides bound to purified HLA-B27. Nature 1991;353:326–9.
12. Kato N, Karaki S, Kashiwase K, Muller C, Akaza T, Juji T, et al. Molecular analysis of HLA-B39 subtypes. Immunogenetics 1993; 37:212–6.
13. Tanabe M, Sekimata M, Ferrone S, Takiguchi M. Structural and functional analysis of monomorphic determinants recognized by monoclonal antibodies reacting with HLA class I α3 domain. J Immunol 1992;148:3202–9.
14. Kikuchi A, Sakaguchi T, Miwa K, Takamiya Y, Rammensee H-G, Kaneko Y, et al. Binding of nonamer peptides to three HLA-B51 molecules which differ by a single amino acid substitution in the A-pocket. Immunogenetics 1996;43:268–76.
15. Sakaguchi T, Ibe M, Miwa K, Kaneko Y, Yokota S, Tanaka K, et al. Binding of 8-mer to 11-mer peptides carrying the anchor residues to slow assembling HLA class I molecules (HLA-B*5101). Immunogenetics 1997;45:259–65.
16. López-Larrea C, Gonzalez-Roces S, Alvarez V. HLA-B27 structure, function, and disease association. Curr Opin Rheumatol 1996;8:296–308.
17. Boisgérault F, Tieng V, Stolzenberg MC, Dulphy N, Khalil I, Tamouza R, et al. Differences in endogenous peptides presented by HLA-B*2705 and B*2703 allelic variants: implications for susceptibility to spondylarthropathies. J Clin Invest 1996;98:2764–70.
18. Wen J, Wang J, Kuipers JG, Huang F, Williams KM, Raybourne RB, et al. Analysis of HLA-B*2705 peptide motif, using T2 cells and monoclonal antibody ME1. Immunogenetics 1994;39:444–6.
19. Tanigaki N, Fruci D, Vigneti E, Starace G, Rovero P, Londei M, et al. The peptide binding specificity of HLA-B27 subtypes. Immunogenetics 1994;40:192–8.
20. Galocha B, Lamas JR, Villadangos JA, Albar JP, López de Castro JA. Binding of peptides naturally presented by HLA-B27 to the differentially disease-associated B*2704 and B*2706 subtypes, and to mutants mimicking their polymorphism. Tissue Antigens 1996; 48:509–18.
21. Simmons WA, Summerfield SG, Roopenian DC, Slaughter CA, Zuberi AR, Gaskell SJ, et al. Novel HY peptide antigens presented by HLA-B27. J Immunol 1997;159:2750–9.
22. Fiorillo MT, Meadows L, D'Amato M, Shabanowitz J, Hunt DF, Appella E, et al. Susceptibility to ankylosing spondylitis correlates with the C-terminal residue of peptides presented by various HLA-B27 subtypes. Eur J Immunol 1997;27:368–73.
23. Tieng V, Dulphy N, Boisgérault F, Tamouza R, Charron D, Toubert A. HLA-B*2707 peptide motif: Tyr C-terminal anchor is not shared by all disease-associated subtypes. Immunogenetics 1998;47:103–5.
24. Garciá F, Marina A, López de Castro JA. Lack of carboxyl-terminal tyrosine distinguishes the B*2706-bound peptide reper-

toire from those of B*2704 and other HLA-B27 subtypes associated with ankylosing spondylitis. Tissue Antigens 1997;49:215–21.
25. López-Larrea C, Sujirachato K, Mehra NK, Chiewsli P, Isarangkura D, Kanga U, et al. HLA-B27 subtypes in Asian patients with ankylosing spondylitis: evidence for new associations. Tissue Antigens 1995;45:169–76.
26. Ren EC, Koh WH, Sim D, Boey ML, Wee GB, Chan SH. Possible protective role of HLA-B*2706 or ankylosing spondylitis. Tissue Antigens 1997;49:67–9.
27. Nasution AR, Mardjuadi A, Kunmartini S, Suryadhana NG, Setyohadi B, Sudarsono D, et al. HLA-B27 subtypes positively and negatively associated with spondyloarthropathy. J Rheumatol 1997;24:1111–4.
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 determining susceptibility to ankylosing spondylitis. Eur J Immunol 1995; 25:3199–201.
30. Khan MA, Kushner I, Braun WE. B27-negative HLA-Bw16 in ankylosing spondylitis [letter]. Lancet 1978;1:1370–1.
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 markers in 158 British patients. Ann Rheum Dis 1986;45:464–74.
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 1992;51:73–7.
33. Kobayashi S, Tanaka M, Tamura N, Hashimoto H, Hirose S. Reactive arthritis induced by tonsillitis [abstract]. Arthritis Rheum 1992;35 Suppl 9:S244.
34. Kobayashi S, Tamura T, Ichikawa G, Takasaki Y, Hashimoto H. Reactive arthritis induced by Pseodomonas aeruginosa. Clin Rheumatol 1996;15:516–20.
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 1996;39:1768–9.
36. Awada H, Baddoura R, Naman R, Klayme S, Mansour I, Tamouza R, et al. Weak association between HLA–B27 and the spondylarthropathies in Lebanon. Arthritis Rheum 1997;40:388–9.
37. Robinson WP, van der Linden SM, Khan MA, Rentsch H-U, Cats A, Russell A, et al. HLA-Bw60 increases susceptibility to ankylosing 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 1997;40:1823–8.
39. Falk K, Rötzschke O, Takiguchi M, Gnau V, Stevanović S, Jung G, et al. Peptide motifs of HLA-B58, B60, B61 and B62 molecules. Immunogenetics 1995;41:165–8.
40. Sidney J, Grey HM, Kubo RT, Sette A. Practical, biochemical and evolutionary implications of the discovery of HLA class I supermotifs. Immunol Today 1996;17:261–6.

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.