The reasons for the association of the human major histocompatibility complex protein HLA-B27 with spondyloarthropathies are unknown. To uncover the underlying molecular causes, we determined the crystal structures of the disease-associated B*2705 and the nonassociated B*2709 subtypes complexed with the same nonapeptide (GRFAAAIAK). Both differ in only one residue (Asp<sup>116</sup> and His<sup>116</sup>, respectively) in the F-pocket that accommodates the peptide C terminus. Several different effects of the Asp<sup>116</sup>→His replacement are observed. The bulkier His<sup>116</sup> induces a movement of peptide C-terminal pLys<sup>9</sup>, allowing the formation of a novel salt bridge to Asp<sup>77</sup>, whereas the salt bridge between pLys<sup>9</sup> and Asp<sup>116</sup> is converted into a hydrogen bond with His<sup>116</sup>. His<sup>116</sup> but not Asp<sup>116</sup> adopts two alternative conformations, one of which leads to breakage of hydrogen bonds. Water molecules near residue 116 differ with regard to number, position, and contacts made. Furthermore, F-pocket atoms exhibit higher B-factors in B*2709 than in B*2705, indicating an increased flexibility of the entire region in the former subtype. These changes induce subtle peptide conformational alterations that may be responsible for the immunobiological differences between these HLA-B27 subtypes.

Major histocompatibility complex (MHC)<sup>1</sup> class I molecules are heterotrimERIC complexes consisting of a heavy chain (HC), β<sub>2</sub>-microglobulin (β<sub>2m</sub> or “light chain”), and a peptide (commonly 8–11 amino acids in length) and are located on the surface of most nucleated cells. Their biological function is to present peptides to a variety of ligands, in particular T cell receptors (TCR) residing on the surface of cytotoxic T cells (CTL).

The human MHC class I molecule HLA-B27 is strongly associated with spondyloarthropathies, a group of closely related inflammatory arthritic diseases; the most common of which is ankylosing spondylitis (AS). About 95% of the AS patients express HLA-B27, although the frequency of this antigen in Caucasians is below 10% (1–3). The association of AS and HLA-B27 was recognized already in 1973 (4, 5). However, the causative role of HLA-B27 in spondyloarthropathies is still not understood, although various studies confirmed that the HLA-B27 molecule itself is the strongest predisposing factor for disease pathogenesis (6).

A number of theories explaining the pathogenic role of HLA-B27 have been proposed (7–9) of which the “arthritogenic peptide” theory and the “misfolding” hypothesis are perhaps the most popular. According to the former, particular properties of its peptide binding pocket enable the HLA-B27 antigen to present specific arthritogenic self-peptides with high sequence homology to peptides of bacterial or viral origin. A peptide with the properties expected for a potential arthritogenic molecule has recently been described (10). In contrast, the misfolding hypothesis relies on an intrinsic tendency of the HLA-B27 heavy chain (HC) to misfold. It has been suggested that the unpaired Cys<sup>67</sup> could facilitate the occurrence of HC homodimers (11), and residues forming the B-pocket have been shown to be responsible for an increased misfolding tendency of the HC in the endoplasmic reticulum (reviewed in Refs. 9 and 12). Accumulation of such misfolded HC molecules could contribute to trigger inflammation via an endoplasmic reticulum stress response. Following this hypothesis, the association of HLA-B27 with spondyloarthropathies would not depend on the peptide-binding abilities of this molecule or its surface properties.

Because of the pronounced disease association, HLA-B27 was among the first MHC molecules whose structures were elucidated (13, 14). The structure (PDB entry 1hsa) provided a composite model for the bound peptide, because the crystallized material contained a mixture of naturally occurring peptides. The electron density in the binding groove was modeled as Ala-Arg-(Ala)<sub>3</sub> (ARA<sub>3</sub>), and the peptide was bound by six pockets (A to F). The most pronounced feature of the HLA-B27 structure was the finding of a B-pocket ideally suited to accommodate Arg at peptide position 2 (p2). Arg at p2 has been confirmed as the primary anchor residue for HLA-B27 in several independent studies (15, 16).
Among the more than 20 different HLA-B27 subtypes that have been identified to date (17), there are two (B*2706 and B*2709) for which only a very limited association with spondyloarthopathies has been proven (18–20). When compared with B*2704 or B*2705, respectively, these subtypes differ only by two (residues 114 and 116, B*2706) or one (residue 116, B*2709) amino acids (1, 7). These changes are expected to affect the epitopes present on the molecule, and the peptide C terminus, suggesting a direct role in the peptide presenting function of HLA-B27 that might lead to differential recognition of the subtypes by cellular ligands such as TCR molecules. This could in turn influence the susceptibility to arthritic diseases. Because they differ in only a single HC amino acid (Asp<sup>116</sup> in B*2705 and His<sup>116</sup> in B*2709, respectively), B*2705 and B*2709 appear to be a promising pair of subtypes to verify any of the hypotheses put forward to explain the association with spondyloarthopathies.

Here we present the crystal structures of the two subtypes bound to the same defined peptide as well as homology models of B*2705 and B*2709 complexed with a peptide derived from Epstein-Barr virus that has been shown to cause differential CTL responses (21).

**EXPERIMENTAL PROCEDURES**

**Protein Preparation**—A plasmid encoding β<sub>m</sub> was a gift from D. C. Wiley (Harvard University, Cambridge, MA). The extracellular region of the HC of HLA-B*2709 was amplified from the CIR-B*2709 cell line (a gift from R. Sorrentino, Università La Sapienza, Rome, Italy) using the primers B27for (GGC GCG ATT TCA GGA AGA TTA AAT GGG CTC TCA CTC CAT GAG GTA T) and B27rev (CGC GAA GCT TTT TAT ACG CCT CCC TTC TCA GGA G). After EcoRI/HindIII digestion, the PCR product was ligated into pHn1 (22). The B*2705 construct was generated by in vitro mutagenesis from B*2709 using the primer TACACCGAGGCGCTACGAGC. HC and β<sub>m</sub> were expressed separately in Escherichia coli as inclusion bodies. The nonamer peptide m9 (GRFAAAIAK) was synthesized by standard solid phase methods (Alta Bioscience, Birmingham, UK) and purified by high-performance liquid chromatography. Protein complexes were refolded from solubilized inclusion bodies as described previously (23). Deviating from the original procedure, inclusion bodies were purified using gel filtration under denaturing conditions (6 M urea), and renaturation was allowed to proceed for up to 14 days at 4 °C. Progress of refolding was monitored by daily analytical gel filtration. Refolded complexes were purified by gel filtration and concentrated to 14–20 mg/ml. N-terminal sequencing of the complex subunits revealed the loss of the N-terminal Met<sup>4</sup> of the HCs of both B27 subtypes, whereas approximately 80% of the β<sub>m</sub> molecules still possessed Met<sup>0</sup>.

**Crystallization and Data Collection**—All crystallization trials were performed by vapor diffusion using the hanging drop method. Initial conditions were identified for HLA-B*2709 in complex with a decameric peptide (RRLRHGHNQV)<sub>(s10)</sub> in a PEG 8000-pH screen. Cross-seedings of drops made from 1 μl of purified using gel filtration under denaturing conditions (6 M urea), and 1 μl of reservoir (22–30% PEG 8000, 100 mM Tris, pH 8.0) produced thin long plates of HLA-B*2705 m9, which were optimized by streak seeding. A data set complete to 2.07 Å was collected from one crystal at 100 K (30% PEG 8000/5% PEG 400 as cryoprotectant) with a MAR charge-coupled device detector using the microfocus X-ray source ID14-4 at ESRF, Grenoble. The space group was P2<sub>1</sub>2<sub>1</sub>2<sub>1</sub>, with one complex per asymmetric unit. Further data collection statistics are given in Table I.

Plate-like crystals of B*2709 m9 were obtained by cross-seeding with B*2705 m9 and subsequent streak-seeding in drops made from 1 μl of protein (20 mg/ml in 20 mM Tris, pH 7.5, 150 mM NaCl) and 1 μl of reservoir (22–30% PEG 8000, 100 mM Bicine, pH 9.0). A data set complete to 1.90 Å was collected from one crystal (300 × 150 × 40 μm<sup>3</sup>) at 100 K (30% PEG 8000/5% glycerol as cryoprotectant) at beam line ID4-4 at ESRF, Grenoble, using an ADSC Quantum 4 charge-coupled device detector. The crystal form is isomorphous to that of B*2705 m9 (see also Table I).

**Structure Determination and Refinement**—The structure of HLA-B*2705 m9 was determined by Molecular Replacement using AMoRe (24) from the CCP4 program suite (25) with HLA-B*2705 m9 (PDB entry 1hsa) as search model but with the ARA-β<sub>m</sub> peptide omitted. Refinement was carried out in two steps. First, refinement with CNS (26) using energy minimization, grouped B-factor refinement, torsion angle-simulated annealing, and individual B-factor refinement alternating with cycles of manual rebuilding using O (27) reduced the R-factor from 0.419 to 0.207 (R<sub>free</sub> = 0.427–0.292). During the last cycles, the peptide was modeled, and water molecules were gradually introduced. In the second step, TLS refinement (28) was carried out using REFMAC (version 5.0 (29, 30), which improved the R-factor to 0.191 (R<sub>free</sub> = 0.249). The final model contains all 276 residues of the heavy chain, all 100 residues of β<sub>m</sub>, all atoms of m9, and 482 water molecules with all in allowed regions of the Ramachandran plot (PROCHECK) (30). For further statistics see Table I.

Because the crystals of B*2705 m9 and B*2709 m9 were isomorphous, initial phases for the structure of B*2709 m9 were obtained from B*2705 m9 with residue 116 changed to Ala. A single round of rigid body refinement followed by simulated annealing and energy minimization was performed with CNS (26) to remove model bias. 2<sub>F</sub> – F<sub>c</sub>, F<sub>c</sub> – F, and simulated annealing omit maps then clearly showed density for a histidine at position 116. After manual rebuilding of incorrectly positioned side chains, water molecules were added using ARP/WARP (31). Subsequent isotropic refinement with REFMAC resulted in 1.09-Å R<sub>factor</sub> converged at R = 0.181 (R<sub>free</sub> = 0.189). Iterative rounds of manual rebuilding using O (32), incorporation of multiple conformations, and maximum likelihood refinement with REFMAC, including restraints for hydrogens and anisotropic B-factor refinement decreased the R-factor to 0.130 (R<sub>free</sub> = 0.154). Several acidic side chains still showed high values of negative difference density at this stage in refinement. As for the disulfide bridges, this phenomenon was attributed to radiation damage (decarboxylation) and was modeled with occupancies of 0.7–0.8 for the affected carboxyl groups until the negative density disappeared at a contour level of 3σ. Refinement converged at R = 0.121 (R<sub>free</sub> = 0.148). The resulting model for B*2709 m9 comprises all 276 residues of the HC, 100 residues of β<sub>m</sub>, with 37 protein residues in multiple conformations, all atoms of m9, 2 glycerol, and 559 water molecules. For further statistics, see Table I.

Superimpositions were performed using O (33), Bragg (33), and the McLachlan algorithm as implemented in ProFit (34). For the r.m.s.d. values given in Table II, the superimpositions were carried out by comparing the C<sub>α</sub> atoms of only the respective regions. The graphics were generated using Ribbons (35), Molscript (36), Bobscript (37), and Raster3D (38).

**Homology Modeling**—Homology modeling was performed to obtain structure models for the complexes B*2705 m9–Ip and B*2709 m9–Ip. The peptide was modeled on the basis of the m9 peptide with fixed Cα positions, and the side chains were changed to the sequence RRWRWRRTY (designated “Ip” hereafter). Subsequently, the conformations of residues 1 and 3 were changed to those observed in B*2709–s10 (PDB entry 1JGD) (2), because s10 has the same N- and C-terminal residues as Ip. After inserting the peptide into the x-ray model of B*2705 m9, the conformations of side chains 3–8 were adjusted by optimizing interactions within the peptide binding groove. Water molecules located closer than 2.5 Å to the introduced peptide side chains were removed. Finally, the side chains of Trp<sup>167</sup> and Arg<sup>26</sup> were changed to those conformations found in the B*2709–s10 complex to allow for the same Arg–Arg–Trp stacking as found in the x-ray structure. After adding polar hydrogen atoms and fixing the potentials, the model was relaxed by 2,500 steps of steepest descent and conjugate gradient energy minimization using the consistent valence force field (39) followed by molecular dynamics started at 300 K with 10,000 steps of 2 fs each. During dynamics calculations all atoms were allowed to move. For all calculations, Insight 2000 (Molecular Simulations, San Diego) was used.

**Protein Data Bank Accession Codes**—Atomic coordinates and structure factors have been deposited in the Protein Data Bank with the accession codes 1JGE (HLA-B*2705 m9) and 1KSN (HLA-B*2709 m9).

**RESULTS**

**Peptide Choice and Structure Determinations**—The heavy chains of both HLA-B*2705 and B*2709 were expressed as inclusion bodies in Escherichia coli and refolded in the presence of β<sub>m</sub> and peptide. Successful refolding was critically dependent on the degree of purity (>95%) of the HC inclusion bodies and the low peptide inclusion bodies resulted in...
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Fig. 1. Representative section of the 2F - F electron density map of B*2709-m9 at 1.09 Å contoured at 1.5σ. The figure shows the conserved pentagonal hydrogen bonding network (indicated with dotted lines), which fixes the N terminus of the peptide to the binding groove.

high levels of aberrantly folded complexes or multimers and disulfide-bridged β₂µ dimers (details will be published elsewhere). A number of peptides that readily bound to empty, cell surface-expressed B*2705 and B*2709 molecules (39) failed to produce sufficient amounts of correctly refolded protein. Finally, a nonapeptide (GRFAAAIAK, hereinafter abbreviated as m9) was designed using the MHCPEP data base (40) that supported refolding of both subtypes, an important prerequisite for a comparative study. This peptide exhibits Arg as the established anchor residue at p2 and a C-terminal Lys, which supported refolding of both subtypes, an important prerequisite for a comparative study. This peptide exhibits Arg as the established anchor residue at p2 and a C-terminal Lys, which acts as an auxiliary anchor at least for B*2705. Furthermore, we took care to avoid destabilizing effects in other positions by choosing small side chains.

Crystals of B*2705-m9 and B*2709-m9 (both of space group P2₁,2₁,2₁) were grown, and diffraction data were collected using synchrotron radiation. Both structures were determined by molecular replacement and refined to R_{value} factors of 0.249 (B*2705-m9, 2.10-Å resolution) and 0.148 (B*2709-m9, 1.09-Å resolution). Fig. 1 shows a representative section of the electron density map of B*2709-m9. For further statistics, see Table I. Overall Fold—The overall fold of HLA-B27 is depicted in Fig. 2A. Both subtypes show the characteristic structural features of an MHC class I molecule, with the HC folded into domains Α₁, Α₂, and Α₃. The Α₃ domain and β₂µm feature immunoglobulin folds. The peptides are bound in an extended conformation in the binding groove formed by the two helices Α₁ and Α₂ and by a floor characterized by an eight-stranded anti-parallel β sheet. Superimposition of the new structures with that of B*2705-ARA₇ reveals that the backbones are virtually identical, with overall r.m.s.d. values below 1 Å (see Table II for details). The subtypes complexed with the same m9 peptide are even more similar with an r.m.s.d. of 0.35 Å, despite the differing F pockets (see below).

Comparison of Peptide Conformations in B*2705 and B*2709—Difference electron density maps showed well-defined density in the peptide binding grooves into which the individual m9 peptides could be modeled without ambiguity. A comparison of the peptide Cᵣ traces is given in Fig. 2B. In both subtypes, the peptide m9 binds in a conformation essentially identical to that observed for the composite nonapeptide model ARA₇ (14). The Arg residue at p2 shows an identical conformation in all structures, emphasizing its importance as the primary anchor.

If the susceptibility to spondyloarthropathies were connected to T cell signaling or recognition by receptors on natural killer (NK) cells, we anticipated that the structures of B*2705 and B*2709, displaying the identical peptide m9, might reveal subtype-specific, significantly different peptide presentation. However, at first sight the structures do not show pronounced differences. When superimposing the peptide binding grooves (residues 1–180), the largest Cᵣ deviation within putative TCR epitopes (center parts of peptides and helices) is only 0.6 Å (Fig. 3A) and located in the center of the peptides at pAla₆. This observation is independent of the superimposition scheme employed (superimposition based either on the β sheet floor, the helices, the complete groove, or the full heavy chain). The neighboring residues pAla₄ to pAla₆ occupy slightly differing positions between the subtypes as well. The only other region, exhibiting similar deviations (maximum, 0.4 Å) comprises residues Asp₇₄ to Arg₇⁹ of the Α₁-helix, close to the F-pocket, as revealed by a superimposition based on the β-sheet floors. Judging from positional errors calculated for the entire structures, the differences observed for the regions around pAla₄ and Asp₇₄ to Arg₇⁹ are small but significant (see Table II). As indicated by the elevated temperature factors in this section of the peptides, the region around pAla₅ is also the most flexible (Fig. 3, C and D), suggesting that the positional errors determined for the central portion of the peptides are larger than those calculated for the complete structures. This is particularly obvious for the B*2709 subtype, where the B-factors of residues located within the A- and B-pockets are very similar to those of the B*2705 structure but differ clearly from those around pAla₅.

Furthermore, in the high resolution structure of B*2709-m9, the side chain of pIle₇ is partly disordered. The conformation incorporated into the coordinates is different from that found in the B*2705-m9 complex (Fig. 3, B and C) and appears to be stabilized by hydrophobic interactions to Trp₁⁴⁷. A second conformation identical to that found in B*2705 was not incorporated into the coordinates, because it could not be modeled without ambiguity. It remains to be determined whether the disorder of this residue can influence the binding of a TCR, because its side chain points toward the binding groove and not to the surface of the molecule.

A comparison of the conformations of m9 and ARA₇ in B*2705 (this study and PDB entry 1hsa) reveals even larger positional differences (up to 1 Å), although the subtype is the same. However, a direct comparison is rendered difficult for a variety of reasons, among them being different crystallization conditions, diverging data collection procedures (4 °C for 1hsa), different space groups and molecular packing (triclinic in 1hsa), and a mixture of peptides in 1hsa, leading to sample heterogeneity and model incompleteness. Nevertheless, analysis of the B-factors of 1hsa also demonstrates that the N-terminal part of the peptide mixture is more rigid than the remaining peptide residues.

Peptide Coordination in the F-pocket—The amino acid characteristic of each subtype (Asp₁¹⁶ in B*2705, His¹¹⁶ in B*2709) is located at the floor of the F-pocket, which accommodates the side chain of the peptide C terminus. With pLys₂, the model peptide m9 features one of the C-terminal residues preferred by B*2705 as demonstrated by a comparative peptide binding study (41). Interestingly, a C-terminal Lys was not found among peptides eluted from B*2709 in vivo (42). The structures of B*2705-m9 and B*2709-m9 show that the hydrophobic butyl group of the side chain of pLys₂ is accommodated in a deep pocket with hydrophobic walls formed by Leu₈₁ (α₁-helix), Leu₈₃ and Tyr₁⁵⁰ (β-sheet floor), and Trp₁⁴⁷ (α₂-helix) (Fig. 4A, for clarity, not all F-pocket residues are shown in the figure). For steric reasons, the bulkier His¹¹⁶ forces pLys₂ to move by 1.1 Å toward the α₁-helix when compared with Asp₁¹⁶ (Fig. 4A), and residue 116 also influences the position of Lys₇⁹ according to subtype (a shift of 1.1 Å). These structural alterations are confined to the floor of the F-pocket in the respective subtype, and it consequently appears unlikely that these residues can be
contacted by any of the protein ligands of HLA-B27 molecules. In addition, the salt bridge connecting pLys9 and Asp116 in B*2705 was not retained in B*2709. Instead, the above-mentioned positional shift of the peptide C terminus leads to the formation of a new salt bridge between pLys9 and Asp77. In B*2705, these amino acids are connected only by hydrogen bonds, via two water molecules (W30 and W147, Fig. 5A). Furthermore, the comparison of B-factors of residues within the two F-pockets (Fig. 5B) shows that those of the B*2709 subtype are larger.

Overall, these distinctive features of F-pocket architecture and characteristics of B*2709 might contribute to a weakening of the interactions that anchor pLys9. Because F-pocket design alone does not provide an obvious explanation for the observed biological differences between the subtypes, we investigated the water networks within the two binding grooves and their possible contribution to the slightly differing modes of peptide presentation.

Water Networks and Their Influence on Surface Properties—The crystal structures of both B*2705-m9 and B*2709-m9 allow a direct comparison of the water networks to identify differences that might be correlated with the single amino acid exchange in position 116. Due to the higher resolution (1.09 Å), more water molecules can be identified in the electron density map of B*2709-m9 when compared with that of B*2705-m9 (2.1 Å). As expected, the pentagonal network of hydrogen bonds within the A-pocket, which is typical for all HLA class I molecules, fixing the peptide N terminus through Tyr5, Tyr9, Tyr11, and a water molecule (W37 in B*2705, W97 in B*2709), is retained in both structures (Fig. 1).

In contrast to the N-terminal part of the peptide, the water networks near its C-terminal section and thus near HC residue 116 differ significantly between the two subtypes (Table III and Fig. 5A). First, the presence of Asp/His116 affects the conformation of pLys9, Lys70, the connected water molecules, and, in turn, the coordination of p5, p6, and p7. The 1.3-Å positional shift of Lys70 between the subtypes (see above) is reflected by the positions of water molecules W212 (B*2705) and W447 (B*2709), respectively. In addition, Lys70 is connected to pLys9 via a central water molecule (W212/W447) in either subtype. This configuration results in denser water and hydrogen bonding networks in the F-pocket of B*2705-m9 as compared with B*2709-m9 (Fig. 5A) and apparently triggers the subtle variations in peptide conformation, because the peptide oxygens of pAla6 and pLe7 are in hydrogen bonding contact with these water molecules. However, the contact of pAla6 to residues forming the F-pocket differs in both subtypes: this amino acid is either connected with W105 (in B*2705), which contacts the central W91, or to W413 (B*2709), which lacks this direct contact with the F-pocket, because a water molecule corresponding to W91 is missing in the latter subtype (Fig. 5A). In addition, His116 adopts two alternative conformations of which one (conformation B) leads to an additional weakening of the network in B*2709, because no hydrogen bond can be formed to pLys9, and to W447 (Fig. 5A). Therefore, in conformation B, His116 retains only one of the three hydrogen bonds by which it is connected when in conformation A (Fig. 5A and Table III).

In the binding groove of B*2709-m9, the water cluster below the peptide and in the proximity of the F-pocket includes twelve water molecules, enclosing a surface of 645 Å2. In contrast, the B*2705-m9 water cluster is formed by thirteen water molecules but nonetheless exhibits a surface of only 625 Å2 (Fig. 5B), reflecting a higher density/tighter packing of the water network in B*2705-m9. Despite these subtype-dependent differences in the interior of intact HLA-B27 molecules, the
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The two B*2705 and B*2709 subtypes investigated here provide a unique opportunity to study the causes underlying the association with spondyloarthropathies. It is highly unlikely that environmental or population-specific factors could be responsible for the differential association (18, 43). Therefore, it might be expected that a detailed comparative analysis should provide clues to understand their distinct pathogenetic roles.

This comparison was facilitated by the high resolution (1.09 Å) available for the B*2709 subtype.

The crucial F-pocket residue 116 of HLA-B27 molecules can either be Asp (as e.g. in B*2705), His (B*2709), or Tyr (as e.g. in B*2706). Whereas B*2705 has been shown to bind in vivo peptides with C-terminal basic, aromatic, and aliphatic residues, only peptides with a nonpolar aliphatic or aromatic (Phe) C terminus have been eluted from B*2709 (42, 44). The most likely reason for these differences is the negative charge of Asp116 in B*2705 and the larger size of the uncharged His116 in B*2709. It is important to note that peptides containing non-preferred residues such as Lys or Tyr as C terminus may also bind to B*2709, even allowing x-ray crystallographic analyses (Lys, this study; Tyr, PDB entry 1JGD2). However, on the basis of reactivity with allospecific CTL, B*2709 exhibits the highest degree of antigenic similarity to B*2705 among all subtypes tested (43).

In any case, B*2709 can reasonably be assumed to have lost a number of CTL epitopes, which are present on B*2705 molecules (21, 42, 43). Differential peptide binding specificities of the subtypes provide an obvious explanation for differential association to spondyloarthropathies. In addition, the recent study by Fiorillo and coworkers (10) demonstrates that thymic T cell selection against the same autoantigen can be influenced by the subtype presenting the peptide, another clear indication that the surfaces of both types of molecules can be distinct. All these effects must be regarded as a consequence of F-pocket variations, because the subtypes differ only at residue 116. The importance of this residue, not only in studies of HLA-B27 molecules, is also highlighted by the finding that mismatches involving this position during bone marrow transplantation from unrelated donors increase both the risk for acute graft-versus-host-disease as well as the risk for transplant-related mortality (45). Furthermore, a Tyr116Ser replacement in HLA-B*3501 reduces the affinity of the molecule for some peptides but increases its affinity for others (46). Mutations of further residues forming the F-pocket have no effect on recognition of the complexes by CTL (47), again pointing to the central importance of this position. Therefore, the question that we addressed in the present structural analysis was how Asp116 or His116 can influence the presentation of the same peptide by minimally differing HLA-B27 subtypes, creating potentially different T cell epitopes.

A detailed analysis of the F-pockets (Table III and Figs. 4 and 5) reveals five different effects of the Asp116His replacement: (i) The bulkier His induces a movement of pLys9 toward a α-helix, resulting in the formation of a salt bridge to Asp77 and affecting the position of Lys70 indirectly, via a water molecule. (ii) The salt bridge between the side chain of pLys9 and Asp116 is converted into a hydrogen bond with His116. (iii) The water molecules in the vicinity of residue 116 differ with regard to number, position, and hydrogen bonding. (iv) In contrast to Asp116, His116 adopts two alternative conformations, one of which leads to breakage of the hydrogen bonds to the peptide terminus and to a water molecule. (v) Atoms shaping the F-pockets exhibit higher B-factors in B*2709 than B*2705, point-
ing to an increased flexibility of the entire region in the former subtype.

The pLys9(NZ)-Asp77(OD2) salt bridge firmly attaches pLys9 to the β₂α₂ domain and weakens its anchoring to the F-pocket floor. This is not seen in the B*2705 subtype, in which a salt bridge connects pLys9(NZ) with Asp116(OD2). Furthermore, the two conformations observed for His116 contribute to the weakening of pLys9 interactions with the floor of the F-pocket: in conformation B, two hydrogen bonds (to pLys9 and W447) are not formed anymore, only that to W276 is retained (Table III and Fig. 5B). Alternative conformations for F-pocket residues have also been observed in HLA-A2 molecules when adjusting to a suboptimal peptide residue (48): the side chains of Arg97 and Tyr116 move in a concerted fashion (“windshield wiper effect”) from a position pointing toward the peptide N terminus to a position pointing toward the peptide C terminus. In the case of B*2709, the rotamer movements are much smaller, and both Asn97 and His116 point toward the peptide N terminus, resulting in only minor changes of the shape of the F-pocket (Fig. 4A). The same orientation is also present with the corresponding residues of the other subtype, indicating that a lysine at the peptide C terminus can be tolerated by both Asp77 and His116 without the pronounced rotamer changes observed in HLA-A2 molecules.

Interestingly, the position of Lys70 is known to influence the primary anchor preference of the B-pocket (16, 49). In the B*2701 subtype, naturally bound peptides insert either with an Arg or, less frequently, with a Gln into the B-pocket. This altered B-pocket preference is due to an Asp74 to Tyr116 change. Because the distance between Asp74 and p2 (about 16 Å) precludes a direct interaction, Lys70 is thought to mediate an indirect contact by acquiring a different rotamer conformation (49). This is clearly not the case for B*2705 and B*2709, because the salt bridge between Asp74 and Lys70 (14) is conserved (Table III and Fig. 5A). The finding of similar B-pocket conformations in the two

| Fitted pair                        | Cα r.m.s.d. (Å) |                      |
|------------------------------------|----------------|----------------------|
|                                   | All atoms      | Heavy chain          | Peptide binding domains α1 + α2 | β₂ Microglobulin | Peptides          |
| B*2705-ARA-B*2705-m9               | 0.764          | 0.823                | 0.721                          | 0.365           | 0.302             |
| B*2705-ARA-B*2709-m9               | 0.778          | 0.850                | 0.695                          | 0.348           | 0.403             |
| B*2709-m9-B*2705-m9                | 0.345          | 0.331                | 0.321                          | 0.236           | 0.226             |
subtypes investigated here lends also support to the concept
that the Asp$^{116}$ → His change does not influence the B-pockets
of the molecules.

In addition, the alterations at the base of each of the F-
pockets cause the formation of different water clusters beneath
the peptide (Fig. 4B). Because the water cluster in both sub-
types is in contact with the peptide backbone (Fig. 5A), it
appears that the Asp$^{116}$ → His replacement is indirectly re-
sponsible for the small but significant differences between the
peptide conformations. These alterations are most pronounced
for the peptide backbone between pAla$^4$ and pAla$^6$, with a
maximum at pAla$^5$ (0.6 Å), whereas the most affected side
chain is that of pIle$^7$. The two subtypes exhibit different rota-
mer conformations for this residue, and the B-factors differ
significantly as well. This result is similar to the situation with
HLA-A2, where a comparative study of several crystal struc-
tures identified peptide positions p4 to p6 to have the highest
conformational variability (50).

The identified subtle structural differences in defined re-
gions of the peptide and the α1-helix both constitute putative
TCR epitopes. Although it is difficult to predict whether an
approaching TCR will be able to recognize these small surface
changes between the HLA-B27 subtypes, there is clearly a
correlation between the surface properties of the subtypes and
the amino acid exchange in position 116. In the model peptide
employed in our structural studies, all residues involved in the
conformational alterations are lipophilic amino acids. How-
ever, even hydrophobic interactions have been reported to be
crucial in some interactions of HLA class I antigens with TCR.
For example, in the case of HLA-A2 recognition by the Tax
peptide-specific A6 TCR, it has been demonstrated that besides
two basic residues (Arg$^{65}$, Lys$^{66}$), Ala$^{69}$ influences the binding

**Fig. 5.** A, detailed F-pocket architecture. Stereo representation of interacting residues and water molecules in the F-pocket. Hydrogen bonds are
depicted with *thin black lines*, salt bridges with *thick green lines*. A more dense bonding network is present in the F-pocket of B*2705:*m9.
Alternative conformations A/B of His$^{116}$ are indicated with *arrows*. B, stereo representation of selected residues forming the F-pocket, *color-coded*
by isotropic B-factor. It is evident that this region is much more flexible in the case of B*2709:*m9.
of the A6 TCR. When this residue was replaced by Gly, the TCR dissociation rate increased significantly, and the half-life of the TCR/peptide/HLA-A2 interaction dropped 7.5-fold (51). Therefore, it seems principally possible that even the small surface changes that distinguish B*2705 and B*2709 in complex with the m9 peptide can exert an influence on the recognition by a given T cell clone.

It has also been suggested that interaction of HLA-B27 molecules with ligands other than TCR could be implicated in spondyloarthropathies, among them killer cell immunoglobulin receptors (KIRs) (52). On B*2705 molecules, KIR3DL1 binds to the supertypic Bw4 determinant, which encompasses residues 77–83 of the α1-helix, and in particular Thr80 is thought to confer protection against NK cell lysis (53). Our study makes it

| Contacts | HLA-B27 subtype and His116 conformation |
|----------|------------------------------------------|
| I. Asp116 (OD2)/His116 (NE2) | B*2705 | B*2709, "A" | B*2709, "B" |
| 1. to pLys9 (NZ) | Salt bridge, 2.85 Å | 2.93 Å | — |
| 2. to Lys70 (NZ) | Via W212: 2.85 + 3.15 Å | Via W447: 3.00 + 2.91 Å | — |
| II. pLys9 (NZ) | — | Salt bridge, 2.73 Å | Salt bridge, 2.73 Å |
| 1. to Asp77 (OD2) | — | — | — |
| 2. to W30 (B*2705) or W276 (B*2709) | 2.79 Å | 2.75 Å | 2.75 Å |
| 3. to W147 (B*2705) or W258 (B*2709) | 2.95 Å | 3.47 Å | 3.47 Å |

* The distances given refer to polar contacts between side chains and/or water molecules. If not otherwise indicated, reported contacts are hydrogen bonds. Only hydrogen bonds <3.60 Å are given. W30 and W276 or W147 and W258, respectively, occupy comparable positions in the F pockets of the subtypes.

* —, no contact formed between the atoms.
possible that intact B*2705 and B*2709 antigens complexed with the m9 peptide could be differentially recognized by KIR3DL1, because the two structures differ slightly (0.4 Å) in the surface region accessible to KIR3DL1 (Fig. 2). However, an involvement of this NK cell receptor is not in line with the demonstration that the B*2708 subtype, which lacks the Bw4 determinant and therefore cannot interact with KIR3DL1, is associated with spondyloarthropathies (54). Similar considerations make it also unlikely that Ig-like transcripts, some of which are known to detect the α3-domain of an HLA class I molecule (52 and references therein), could act as distinguishing ligands. However, the present results do not allow the exclusion of a pathogenetic mechanism in which some aberrantly folded forms of HLA-B27 molecules (12, 52) interact with KIR3DL1 or an Ig-like transcript molecule.

Because salt bridges in hydrophobic environments have a higher stabilizing effect on proteins than hydrogen bonds (50), the change of the salt bridge connecting Pyl5 and Asp116 in B*2705 to a hydrogen bond to His116 in B*2709 accounts for the higher B-Factors of residues forming the B*2709 F-pocket. This suggests a tighter binding of the peptide in B*2705 and altered dynamic properties of the entire molecule. Preliminary results from circular dichroism and microcalorimetry studies together with time-resolved fluorescence depolarization experiments of the subtypes confirm these predictions. Differences in dynamic properties might influence the biological behavior of the subtypes (9). It remains to be seen how peptides with more favorable C-terminal amino acids like nonpolar aliphatic or aromatic (Phe) residues, will influence the structures of the subtypes.

Due to the model character of the m9 peptide, we carried out homology modeling of the subtypes with the Imp peptide, for which a differential T cell response according to subtype has already been demonstrated (21). However, these modeling studies are currently inconclusive, because the models of both subtypes reveal no significant structural difference. Our results are in agreement with previous studies (55–57), which all emphasize that even small changes in a TCR epitope are sufficient to trigger different CTL responses. The discrepancy between our homology modeling study and the results from experiments with CTL (21) can only be resolved when further crystal structures, supported by biophysical measurements of the subtypes complexed with a differentially recognized natural peptide, will be available.

In conclusion, our study reveals subtle alterations in surface properties between the disease-associated B*2705 and the non-associated B*2709 subtypes, which are indirectly linked with the Asp116 → His exchange. Although it appears possible that TCR molecules could detect these differences in peptide presentation, thus supporting the arthritogenic peptide concept (55), we can not presently exclude other pathogenetic mechanisms (2, 9, 12, 52) that might also involve noncanonical peptide presentation by HLA-B27 molecules (56, 57). The proposed homodimerization through Cys67 of B*2705 (11), on the other hand, seems to us less likely as a disease-related feature, because B*2709 would be expected to form such dimers as well.

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