4. Discussion

4.1 Discussion of expression and reconstitution experiments

The basic idea behind the experiments described here was to produce *in vitro* properly folded and functionally active HLA-B27 molecules together with peptides. This was obtained for all three subtypes B*2703, B*2704 and B*2706 in complexes with self- and viral peptides, yielding different results. The method described by Garboczi and co-workers (Garboczi et al., 1992) for formation of an HLA-A2:β_2m:peptide complex was successful and provided refolded proteins that were purified with very good quality.

In comparison to the isolation of membrane proteins in their native state the *in vitro* reconstitution has many advantages. *E. coli* cells grow well in LB medium and they accumulate the proteins in the cytoplasm to a very high level (the average amount of 70 mg of protein per one liter of culture). However, the main advantage of *E. coli* expression system is that reconstitution can be achieved with a single peptide.

Inclusion bodies are amorphous aggregates and resistant to proteolysis by *E. coli* proteases, which results in high yield protein production. However, the experiments conducted in this study showed that the yield of inclusion bodies as well as protein quality can vary between the proteins. As already mentioned, B*2706 subtype was less problematic in expression than B*2703 and B*2704. Before initiation of complex formation, all inclusion body proteins were tested for purity by SDS gels (section 3.1.2). Inclusion bodies of best quality were obtained for B*2703 and β_2m, whereas expression of B*2704 and B*2706 resulted in production of less pure proteins. It is difficult to explain why the quality of these two subtypes was reduced in comparison to HC of B*2703 and light chain as well as why the expression level of B*2703 was much lower than that of B*2704 and B*2706.

Successful gene expression in *E. coli* depends on many factors such as codon usage, expression vectors, folding competence, proteases. There are some codons in every species for which only very little tRNA is made. In *E. coli* these are mainly AGA and AGG (Arg codons). It could happen that the amino acids which differentiate B*2703 from B*2704 and B*2706 have lower adaptiveness in *E. coli,* and therefore some subtypes are expressed at lower yield. However, this is not the case here because codons of all polymorphic residues in the three subtypes have a very similar percentage of codon usage. The expression vector was the same for all three subtypes as well as for β_2m, and was also used previously for other HLA-B27 subtypes, and no problems with expression were reported. The folding and stability of mRNA could

influence the gene expression as well, but not in this case since the mRNA folds for all subtypes reveal similar structure in the regions of all mutations characteristic for each subtype (online analysis with mfold by Zuker and Turner).

Complex formation resulted in diverse amounts of properly folded HLA-B27:β_2m:peptide complexes. The yield of B*2703:β_2m:peptide was low (0.4 mg/ml), and the peak obtained after size exclusion chromatography was broad. However, the complex eluted at a position expected according to molecular weight, and an SDS-PAGE analysis confirmed that the ternary complex contains all components. B*2704 gave a proper amount (about 1.3 mg/ml) of complexes in presence of 20 % glycerol after testing different conditions. The HLA-B*2704 complexes, formed with the peptides used here, appear as sharp peaks on gel filtration chromatography at an elution time consistent with the expected molecular weight of the complexes. Analysis of the peaks containing the complexes by SDS-PAGE revealed that they are composed of two polypeptide chains of the expected sizes of the B*2704 heavy chain and β_2m, and delivered complexes of high purity. It is surprising that B*2703 revealed poor complex formation since all three subtypes are closely sequence-related.

Additional experiments on complexes of B*2703 with both peptides performed in the frame of this study showed that the stability of HLA-B*2703:β_2m:pVIPR was higher than that of the complexes of B*2705 and B*2709 with the same peptide, and complex formation of the two last mentioned subtypes was easier than with B*2703. The pVIPR and pLMP2 peptides reported here have been shown to bind to HLA-B2705 and B*2709 and supported the refolding of the complexes (Hülsmeyer et al., 2004; Fiorillo et al., 2005).

4.2 Discussion of crystallization experiments

For all HLA-B27 subtypes (B*2703, B*2704 and B*2706) in complex with individual peptides, crystals suitable for X-ray diffraction were obtained. As described above (chapter 3.2) the crystals of all complexes were obtained by using hanging-drop vapor diffusion. Since all three subtypes are very closely related to B*2705 (ancestral allele) and B*2709, for B*2703, B*2704 and B*2706 we employed crystallization conditions in which crystals of the other two subtypes grew. As expected, only the polyethylene glycol concentration in the precipitant solution had to be modified to obtain crystals large enough for X-ray data collection. Crystals of B*2704:β_2m:peptide had a slightly different morphology (long, thin plates) than the ones of B*2703 and B*2706 (prisms). The shape and size of the crystals depends on internal symmetry and relative growth rates along the various directions of the crystal. Crystals are formed by the

repetition of unit cells in 3-D space. Therefore, the shape of a crystal is partly related to the shape of the unit cell. Different morphologies of crystals implicates that they may have different unit cell parameters.

4.3 Discussion of crystallographic data, structure analysis and structural comparisons

This study was designed and carried out in order to gain better knowledge about HLA-B*27 subtypes and their association to Ankylosing Spondylitis. The influence of A-pocket polymorphism on self- and antigen peptide conformation was investigated using the B*2703 subtype, whereas comparison of pLMP2 and pVIPR binding modes in B*2703, B*2704 and B*2706 was thought to bring valuable information about the relationship of HLA-B27 subtypes and AS. Determining, whether conformations of these peptides bound to B*2704/B*2706 resemble those found in B*2705/B*2709 was performed in order to verify, whether molecular mimicry can be generalized.

4.3.1 How are peptides such as pLMP2 and pVIPR bound to B*2703?

The crystal structures of the B*2703:β_2m:peptide complexes presented in section 3.3.1.1 allowed to elucidate how the two peptides, pVIPR and pLMP2 which were previously investigated in complex with B*2705 and B*2709, are bound to B*2703. Thanks to detailed analysis of peptide conformation in the binding groove, it became possible to establish the maintenance of a pentagonal bonding network within the A-pocket of B*2703.

This study is the first determining the crystal structures of the human MHC class I antigen HLA-B*2703. The pLMP2 and pVIPR peptides are bound to the groove of HLA-B27 in drastically different conformations (Figure 3.14 and 3.15). The peptides exhibit sequence dissimilarities at p3 and from p7 to p9. The structures of B*2703 in complex with pLMP2 and pVIPR show that drastic changes in the binding mode of both peptides are seen in the middle part. This was already observed for two other closely related subtypes, B*2705 and B*2709 (Hülsmeyer et al., 2004; Fiorillo et al., 2005). These two distinct conformations of the peptides are called p4α and p6α (Figure 1.8) depending on the residue of the main chain whose Cα-atom is in α-helical conformation (p4 or p6 respectively; Hülsmeyer et al., 2004; Fiorillo et al., 2005). However, the conformations of the N- and C-termini of both peptides are extremely similar when

bound to B*2703 subtype as well as to B*2705 and B*2709 (Hülsmeyer et al., 2004; Fiorillo et al., 2005). In case of HLA-B27 bound peptides, the p6α conformation has been observed in the structures of B*2705 with pVIPR (Hülsmeyer et al., 2004), pLMP2 (Fiorillo et al., 2005) and pGR (Rückert et al., 2006).

There is so far no explanation for the different binding modes of the two peptides in B*2703, but the differences can have consequences for the recognition of the complexes by TCR molecules since residues p3-p7, which form the central section of the investigated peptides, are thought to be accessible to the T-cell receptor (Stryhn et al., 1996; Sant Angelo et al., 2002; Rudolph and Wilson, 2002). In case of the p6α conformation which was found for pVIPR when bound to B*2703, the middle part of the peptide is buried deeper in the binding groove whereas in the p4α binding mode this section of the peptide pLMP2 is exposed to the solvent, and the interactions with the same receptor will consequently be different. In B*2703:β_2m:pLMP2, the central part of peptide is also more flexible as demonstrated by elevated B factors (Figure 3.16).

The ancestral allele B*2705 is so far the only one where a dual peptide binding mode was elucidated for the pGR (Rückert et al., 2005) and pVIPR peptide (Hülsmeyer et al., 2004). The differences in the structures of pVIPR and pLMP2 in complexes with B*2705 and B*2709 (Hülsmeyer et al., 2004; Fiorillo et al., 2005) can be explained by polymorphic residue 116 in the binding groove. Since the bottom of the binding groove of B*2703 is identical to that in the B*2705 subtype, it is not surprising that the pVIPR binding mode shows high similarities although it was present in only one conformation in B*2703. However, comparison of pLMP2 conformation in complexes with B*2703, B*2704, B*2705, B*2706 and B*2709 brought unexpected result. Overlays of the B*2703:β_2m:pLMP2 structure with those of B*2704, B*2705, B*2706 and B*2709 in complexes with pLMP2 indicate that the peptide backbone conformation is similar in all subtypes except B*2705. However, numerous differences were noticed in side chains of solvent exposed residues, mostly between B*2703 and B*2704 or B*2706. It is difficult to explain this result, especially because the B*2703 subtype was expected to present the pLMP2 peptide in the p6α conformation which was also found for this peptide in B*2705 as well as for the pVIPR in B*2703 and B*2705 subtypes.

4.3.2 How does the His59Tyr replacement in B*2703 affect the binding mode of N-terminal amino acids within the A-pocket?

Comparison of the results obtained for B*2703 in complex with pLMP2 and pVIPR to the crystal structures of B*2705 and B*2709 in complex with these peptides (Hülsmeyer et al., 2004; Fiorillo et al., 2005) permits us to discuss the replacement of the common Tyr59 by His on peptide binding. This exchange in B*2703 seems to have no influence on peptide conformation. It is probably due to the additional water molecule which compensates for the shorter side chain of His59 (B*2703) in comparison to Tyr59 (other B27 subtypes).

Among HLA subtypes the ones with different amino acid exchange in the A-pocket were identified: HLA-B37, HLA-B14, HLA-B18, HLA-B*7301, and the pairs of the HLA-B subtypes HLA-B*5101/HLA-B*5102 and B*3704/B*3701 (Kikuchi et al., 1996, Estefania et al., 2002). B*3704 differs from B*3701 and B*5101 differs from B*5102 just by a replacement of His for Tyr at position 171. Although residue 171 is located in the α-2 helix, it is similar to His/Tyr59, as it lines the A pocket of the peptide binding groove. Due to this exchange in B*5101, the H-bond network within the A-pocket is altered. The alternetive arrangement of the N-terminal part of the peptide results in deeply burried central part of the peptide and change of the epitope for TCR recognition (Maenaka et al., 2000). It is less likely that such a situation will apply to B*2703 subtype since the H-bond network within the A-pocket as well as the N-terminus of both peptides remains unchanged with respect to other HLA-B*27 subtypes complexed with the same peptides.

Solving the two crystal structures of the B*2703 subtype allowed to answer the question whether any structural differences can be observed in the A-pocket as a result of the His to Tyr exchange at position 59 of the HC. The replacement of Tyr59 occurs only in B*2703 (His59) and B*2717 (Phe59), and since Tyr59 is known to take part in a pentagonal network of hydrogen bonding pattern, which is characteristic for MHC class I molecules (Madden, 1995), it was of importance to elicit whether this single amino acid exchange can influence the structure.

The two first residues of both peptides in B*2703 (pArg1 and pArg2) adopt identical positions as in other HLA-B27 subtypes, and pArg2 is known to be the primary residue (Madden at al, 1992; Urban et al., 1994), which is required for peptide anchoring in the HLA-B27 binding groove. The structures of B*2703 with both pVIPR and pLMP2 confirm this finding. The single amino acid exchange in the A-pocket of HC, where pArg1 is bound, does not influence the structure of this interaction. As presented in this study the hydroxyl group of Tyr59

is replaced in B*2703 by an additional water molecule which allows to maintain the characteristic pentagonal hydrogen bond network (Figure 3.18).

This finding was even more interesting in the light of results obtained for the B*5101 subtype. As already mentioned, B*5101 carries an amino acid exchange in the A pocket (Tyr171 to His171). Residue 171 is located at the C-terminus of the α2-helix. The exchange of this otherwise highly conserved amino acid is expected to influence the pentagonal hydrogen bonding architecture at the peptide N terminus as well. Recently, the crystals structures of two peptides (KM1 and KM2) in complex with B*5101 were solved (Maenaka et al., 2000). The structure of B*5101:KM1 (2.2 Å) revealed differences in the hydrogen bonding network in comparison with the one observed for B*2703:β_2m:pLMP2 and B*2703:β_2m:pVIPR. An additional water molecule bound to His59 is mimicking the phenolic group of Tyr171 and permits the formation of a hydrogen bond to the N terminus of the peptide, resulting in an "elevation" of the N-terminal part of the peptide. However, this leads to a non-standard arrangement, resulting in a tetragonal or even trigonal hydrogen bonding network (Figure 4.1).

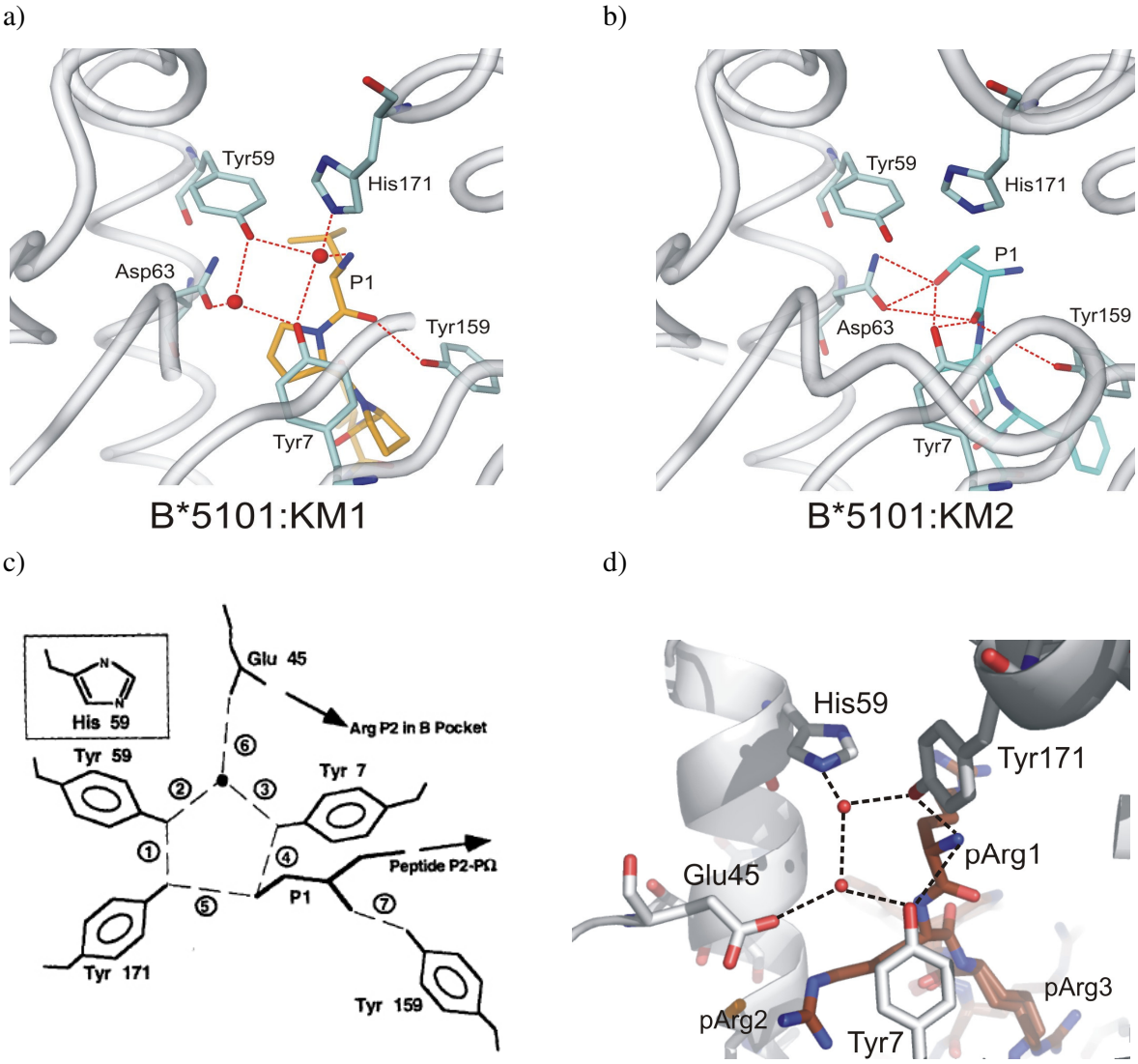

Figure 4.1. Structural comparison of the A-pocket in B*5101 and B*2703. The structure of the B*5101 A-pocket when complexed with a) KM1 and b) KM2 peptides. P1 is the first amino acid of the peptide. Residues which form hydrogen bonds in the A-pocket (Tyr59, His171, Tyr159 and Tyr7) are in stick representation while the rest of the molecule is shown as cartoon. c) The classical hydrogen bonding network within the A-pocket (Griffin et al., 1997), as schematic diagram. Conserved tyrosines at positions 7, 59 and 171 form a pentagonal H-bond network with a water molecule and the N-terminus (P1) of the peptide. The His59 side chain which replaces Tyr59 in B*2703 is shown in the inset. d) Structure of the A pocket in B*2703 subtype in complex with peptide pLMP2. The hydrogen bonding network between conserved residues, water molecule and the carbonyl oxygen of pArg1 is drawn with dashed lines; α1- and α2-helicies are in ribbon representation.

In contrast, when pLMP2 and pVIPR form complexes with B*2703, the hydrogen bond network in the A pocket is arranged in the classical pentagonal way (Figure 4.1 d). The structures described here are in sharp contrast to the structure of B*5101:KM2, where no pentagonal bond network has been conserved (Maenaka et al., 2000). The limited resolution of 3.0 Å for B*5101:KM2 did not permit to model water molecules, precluding a direct comparison with the structures described here.

The crystal structures of B*2703:β_2m:pLMP2 and B*2703:β_2m:pVIPR allowed, for the first time, a detailed analysis of this HLA subtype. In this allele the polymorphism in the A-pocket of the peptide binding groove has no influence on the conformation of the N-terminus of the peptide. This may also be a consequence of the tight sandwich formed by the side chains of Arg62, pArg1 and Trp167 (Hillig et al., 2004). However, it is still not possible to explain different peptide binding modes exhibited by pVIPR and pLMP2 when presented by B*2703. It seems that residue 116 does not determine on its own in which conformation a peptide will occur since in B*2703 the two peptides are bound in drastically different manner. This resembles the situation in B*2705 subtype in complex with pVIPR where both peptide conformations are possible. The distinct conformations, which characterize the binding of pVIPR-p6α and pLMP2 in the B*2705 subtype, are now also seen for B*2703. This result indicates that it is impossible to provide a rationale for this finding only on the basis of a crystallographic analysis.

4.3.3 Thermodynamic properties of B*2703 subtype in complex with pLMP2 and pVIPR peptides.

Performing CD and DSC measurements of HLA-B*2703 complexes with the self-peptide pVIPR and the viral peptide pLMP2 enabled discussion of the results with previously obtained data for two other complexes B*2705 and B*2709 with the same peptides (Misselwitz et al., unpublished). CD and DSC measurements of B*2703 subtype in complex with pLMP2 and pVIPR were performed by co-worker Dr. Rolf Misselwitz. According to CD measurements, pVIPR bound to both subtypes formed slightly less stable complexes (58.4 to 60.8°C) in comparison to B*2703:β_2m:pVIPR (62.3°C). However, complexes of all three B27 subtypes with pLMP2 have an almost identical T_m (49.6 to 51.4°C), much lower in comparison with those found for pVIPR. These finding suggest that the stability of complexes depends on the bound peptide and not on the subtype (Misselwitz et al., unpublished), (Table 4.1).

Table 4.1 Thermal unfolding of B*2703, B*2705, and B*2709 in complex with pVIPR or pLMP2.

Samples	T_m1 (°C)	ΔH_m1 (kcal/ mol)	T_m2 (°C)	ΔH_m2 (kcal/ mol)	T_m3 (°C)	ΔH_m3 (kcal/ mol)	ΔH_{tot} (kcal/ mol)	T_m (CD) (°C)
2703:pVIPR	-	-	62.9±0.3	104±4	66.8±0.2	178±2	282	62.3±0.7
2705:pVIPR	-	-	63.5±0.2	100±7	67.3±0.2	168±3	268	60.8±0.3
2709:pVIPR	-	-	60.2±0.4	114±5	64.1±0.2	173±1	287	58.4±0.6
2703:pLMP2	51.8±0.5	75±7	55.2±0.2	109±4	65.0±0.3	124±8	308	51.4±0.5
2705:pLMP2	52.7±0.5	83±2	57.7±0.5	129±3	64.1±0.1	77±3	289	51.4±0.7
2709:pLMP2	50.4±0.4	80±3	55.1±0.3	127±2	64.3±0.1	78±2	285	49.6±0.5
β_2m[a]	-	-	-	-	63.8±0.5	75±9	-	64.6±0.8

[a] (Hillig et al., 2004)

10 mM phosphate buffer, pH 7.5, 150 mM NaCl; c_{compl} = 3-4 µM, $c_{\beta 2m}$ = 10-12 µM

± - standard deviation

$T_m1/T_m2/T_m3$ – thermal midpoints of transitions 1, 2 and 3; T_m(CD) – the temperature where just one-half of the total ellipticity change is observed; ΔH_m1, ΔH_m2, ΔH_m3, ΔH_{tot} – calorimetric heat changes at the corresponding T_m values determined from the are under the corresponding transition peaks. ΔH values describe the enthalpy change between the folded and unfolded states.

As expected from previously published studies (Hillig et al., 2004; Hülsmeyer et al., 2005), the unfolding of the heterotrimeric HLA-B27 molecules shows a more complex unfolding behaviour in comparison to β_2m. The asymmetry or partial overlapping of the excessive heat capacity curves implicate multi-step unfolding reactions for B*2703:β_2m:pVIPR and B*2703:β_2m:pLMP2 complexes. Dissociation of the peptide and unfolding of the complex can occur sequentially or simultaneously, and unfolding of HC domains can occur separately or independently resulting in more or less separated or partially overlapping melting profiles. Therefore, to obtain more detailed information from the unfolding transitions, the excessive heat capacity curves were deconvoluted (Figure 4.2). Using the two state routine of the ORIGIN software two (B*2703:β_2m:pVIPR) or three (B*2703:β_2m:pLMP2) partially overlapping peaks with T_m1 to T_m3 and corresponding "apparent" enthalpies ΔH_m1 to ΔH_m3 were calculated.

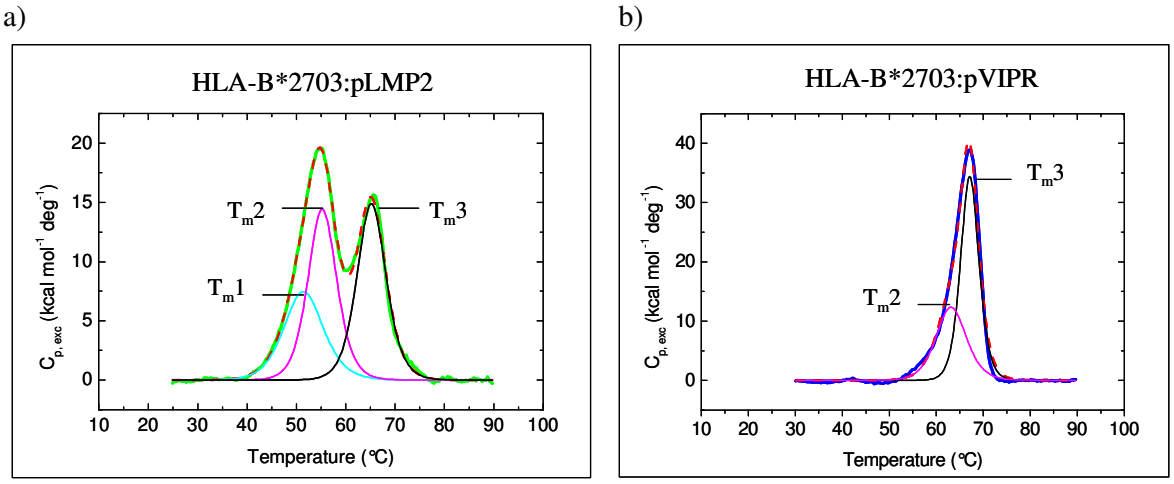

Figure 4.2. Deconvolutions of experimental excessive heat capacity curves of B*2703:β_2m:peptide complexes. a) Deconvolution excessive heat capacity curves of B*2703:β_2m:pLMP2 (green), and b) B*2703:β_2m:pVIPR (blue). Deconvolution (cyan, magenta and black) results in three two-state transitions for B*2703:β_2m:pLMP2 and two two-state transitions for B*2703:β_2m:pVIPR.

As described above, the B*2703:β_2m:pLMP2 complex unfolds in two separated peaks with an asymmetry in the low temperature region of the peak. The first transition is characterized by a melting temperature significantly lower than β_2m, whereas the second one melts at about the T_m of β_2m (64.6°C). Deconvolution of the experimental excessive heat capacity curve yields two overlapping transitions for the low temperature peak (T_m1, ΔH_m1 and T_m2 and ΔH_m2; Table 4.1) and only one for the second, high temperature peak (T_m3, ΔH_m3; Table 4.1). Complexes of B*2705 and B*2709 subtypes with pLMP2 also reveal a three-step unfolding with melting temperatures very similar to those obtained for B*2703:β_2m:pLMP2, but with a significantly reduced ΔH_m3 (not published). ΔH_m3 (about 78 kcal/mol) and T_m3 (about 64°C) values of B*2705:β_2m:pLMP2 and B*2709:β_2m:pLMP2 complexes are similar to that of free β_2m (63.8°C, 75 kcal/mol), corresponding to the melting of folded β_2m (Table 4.1).

The deconvolution results of B*2705/09:β_2m:pLMP2 complexes suggest, that at first two domains of the HC with different energetic profiles unfold (T_m1, ΔH_m1; and T_m2, ΔH_m2) with concomitant dissociation of folded β_2m and unfolding of β_2m at higher temperature (T_m3, ΔH_m3). Energetic domains cannot be unambiguously assigned to known structural domains; however, their melting is characterized by defined T_m and ΔH_m values. In contrast, a significantly higher ΔH_m3 value of 124 kcal/mol was obtained for B*2703:β_2m:pLMP2. Similar to the unfolding of B*2705/09:β_2m:pLMP2 complexes two energetic domains of the HC unfold at first (T_m1, ΔH_m1; and T_m2, ΔH_m2; Table 4.1). The increased ΔH_m3 of 124 kcal/mol can only be explained by an additional enthalpic contribution of the HC to the unfolding enthalpy of β_2m.

In contrast, B*2703 and B*2709 in complex with pVIPR unfold in single peaks with a slight asymmetry at the low temperature side of the excessive heat capacity curves, and unfolding starts at higher temperatures compared to HLA-B27:β_2m:pLMP2 complexes (Figure 4.2 b). HLA-B27:β_2m:pVIPR complexes mostly melted at a similar temperature as free β_2m (B*2709:β_2m:pVIPR) or even at slightly higher temperatures (B*2705:β_2m:pVIPR and B*2703:β_2m:pVIPR). Deconvolution of the melting curves of all three subtypes in complex with pVIPR yielded only two partly overlapping transitions, characterized by T_m2, ΔH_m2 and T_m3, ΔH_m3, with T_m2 and T_m3 differing by about only 4 °C. A correlation of the two partly overlapping transitions (T_m1, T_m2, ΔH_m1, ΔH_m2) with the melting of structural domains (heavy chain and β_2m) was not justified by the data. However, this melting behavior indicates that dissociation of the complexes is coupled to the unfolding of both HC and β_2m (Table 4.1). The higher ΔH_m3 value for HLA-B*2703:β_2m:pVIPR complex, in comparison with isolated β_2m, represents overlapping enthalpy changes resulting from the unfolding of β_2m and parts of the heavy chain that must be a consequence of the Tyr59His exchange between B*2705 and B*2703.

The comparison of melting patterns of B*2703 subtype with B*2705 and B*2709 again points to peptide-dependent dissociation and unfolding of the complexes. The pLMP2 peptide appears to change the unfolding behavior of the investigated subtypes in a way that the pMHC complex becomes less stable and dissociates and unfolds in three steps, whereas complexes with pVIPR always dissociate and unfold in two-steps. For both peptides, the unfolding behaviour is subtype-independent.

4.3.4 Do the polymorphisms in B*2704 and B*2706 influence the binding mode of pLMP2 and pVIPR?

This study describes for the first time the structures of the two HLA-B27 subtypes, HLA-B*2704 and B*2706, both in complex with a self-peptide (pVIPR) and a viral peptide (pLMP2). These nona-peptides were previously investigated with B*2705 and B*2709, and the results allowed to suggest a structural frame-work for molecular mimicry as a cause for disease association (Hülsmeyer et al., 2004; Fiorillo et al., 2005). It was found that a pathogen-derived peptide can be presented by an MHC in a subtype-dependent manner, and the self-antigen (pVIPR) has been shown to mimic a viral peptide in the AS-associated subtype (B*2705). Additionally there is evidence that individuals with the B*2705 subtype possess T cells directed against a self-peptide.

Since the closely related B*2704 and B*2706 form a second pair of subtypes with differential association to AS, a detailed analysis was expected to elucidate whether they present certain peptides in the same manner and give a hint in explaining the basis for the role of antigens specified by the major histocompatibility complex (MHC) in this disease.

Section 3.3.2.1 shows that a pathogen-derived peptide such as pLMP2 as well as self-peptide such as pVIPR can be displayed by two closely related B*2704 and B*2706 subtypes in similar binding modes (Table 4.2). Since B*2704 and B*2706 differ only in two amino acids and are, similarly to B*2705 and B*2709, differentially AS-associated, it was interesting to compare their structures in complex with pLMP2 and pVIPR with the results for the complexes of these peptides with B*2703, B*2705 and B*2709.

Surprisingly, both peptides presented by the B*2704 subtype as well as by B*2706 are in the p4α conformation (Table 4.2).

Table 4.2. pLMP2 and pVIPR peptide conformations in HLA-B27 subtypes. Conformation p4α - main chain φ/Ψ torsion angles in α-helical conformation at p4α, conformation p6α - main chain φ/Ψ torsion angles in α-helical conformation at p6α.

Subtype / Peptide	B*2703	B*2704	B*2705	B*2706	B*2709
pLMP2	**p4α**	**p4α**	**p6α**	**p4α**	**p4α**
pVIPR	**p6α**	**p4α**	**p4α/p6α**	**p4α**	**p4α**

Since there is no difference in amino acid sequence at the floor of the binding groove between B*2704 and B*2705, it is surprising that the B*2704 subtype presents the self peptide only in p4α conformation and not in two modes as in B*2705. In case of B*2706, which differs from B*2705 and B*2709 in five residues, two of which are located at the bottom of the binding groove, it was highly possible that the His114Asp and the Asp116Tyr exchanges would contribute to the pVIPR presentation by the B*2706 subtype.

The influence of the two polymorphic residues located at the bottom of the binding groove (Table 1.1) on overall peptide binding mode can not be established explicitly. Nevertheless, some interactions of residues 114 and 116 with the peptide amino acids might explain the conformation of both peptides, and an influence of other polymorphic residues (outside the groove) in B*2704 and B*2706 can be noticed. p3 of both peptides interacts directly or indirectly with Asp114 of B*2706 and disables the interaction of pArg5 with the binding groove, resulting in pArg5 directed to the solvent. Located in the α2-helix of B*2705/09, Val152 is

neutral and shorter than the negatively charged Glu which occurs at this position in B*2704 and B*2706. Therefore it is likely that Glu152 would form a novel salt bridge with positively charged pArg5 (Figure 4.3 a). However, this is possible only with the pLMP2 peptide since the pArg5 side chain of pVIPR is directed more towards the N terminus of the peptide in the B*2704 subtype (Figure 4.3 b), while in B*2706 it could not be to modeled.

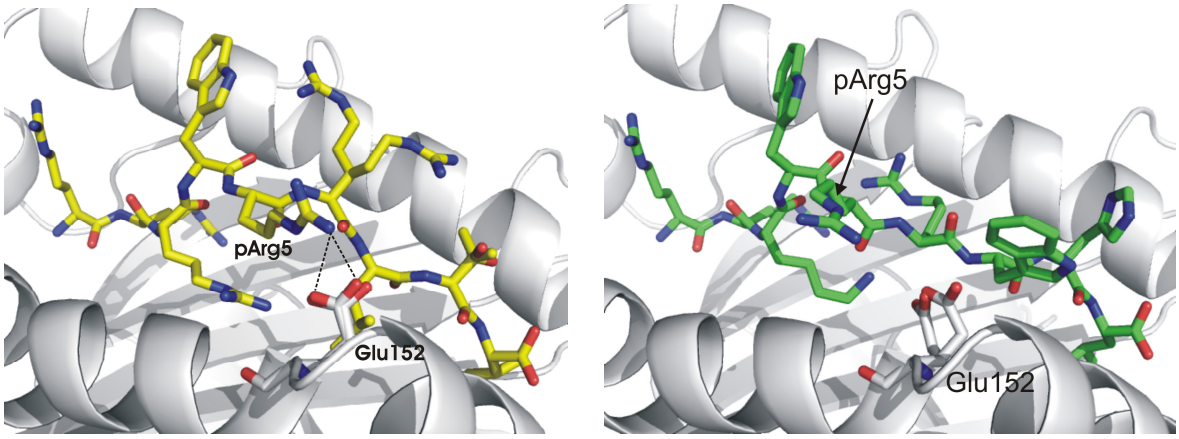

Figure 4.3. Consequences of Val to Glu change in B*2704. a) A novel salt bridge between polymorphic residue Glu152 and pArg5 in the B*2704:β_2m:pLMP2 structure, b) The conformation of pArg5 in B*2704:β_2m:pVIPR structure.

Moreover, Val152 exchanged to a charged Glu is most probably the reason why pTrp7 of the pVIPR peptide in B*2704 and B*2706 is pushed out of the peptide binding groove. The Asp77Ser and Ala211Gly exchanges in B*2704 and B*2706 seem to have no influence on the peptide conformations (Table 3.8 and Table 3.10). However, it has been reported that due to Asp77Ser mutation in H-2K^{bm3} (MHC class I) the TCR β-chain interacts more intimately with the pMHC (Rudolph and Wilson, 2002), which increases the number of contacts between the β-chain and the pMHC. Therefore it cannot be excluded that in the class I alleles B*2704 and B*2706 the Asp77Ser exchange will also influence the peptide recognition by a TCR.

Similar to the structures of B*2705, B*2709 and the recently solved B*2703 with viral peptide, pArg2 serves as primary anchor also in B*2704 and B*2706. It is very interesting that the peptide N-terminus in the structures of both subtypes with pLMP2 and pVIPR is identical in all crystal structures which are compared in this study. However, for the first time a new anchor residue is observed in the structures with pLMP2 and pVIPR peptides. This occurs in B*2706, where pLMP2 is bound by a salt bridge to the polymorphic residue Asp114 through pArg3, establishing peptide residue 3 as a secondary anchor for the first time in HLA-B27 subtypes. In case of B*2706:β_2m:pVIPR, a contact between pLys3 and Asp114 is mediated by a water molecule since the distance between the pLys3 and Asp114 side chains is longer.

As expected, in B*2704 (His114) a comparable situation could not be found. It was already reported that p3 is an important anchor position for peptide binding to HLA-B27 (Sesma et al., 2002). Peptides with pArg3 are overrepresented in B*2706 but not in B*2704 which has a strong preference for aliphatic or aromatic amino acids (Sesma et al., 2002; López de Castro et al., 2004). The occurrence of an additional anchor residue could explain the conformations of the pLMP2 peptide in subtype B*2706. It is possible that the polymorphism at position 114 (His against Asp) causes formation of a salt bridge between Asp114 and pArg3 instead of a salt bridge between Asp116 and pArg5 (found in B*2705:β_2m:pLMP2 and in the p6α conformation of B*2705:β_2m:pVIPR as well as in B*2703:β_2m:pVIPR). Also a direct interaction between pArg5 and residue 116 (Tyr in B*2706), described in B*2705:β_2m:pLMP2 (Fiorillo et al., 2005) is not possible in B*2706 since p5 interacts with Glu152, additionally the bottom of the binding groove is already filled by p3.

One more important similarity was noticed when all HLA-B27 subtypes in complexes with both peptides were compared, namely in all complexes with the viral peptide pLMP2 the C-terminus of the peptide sits slightly deeper inserted in the binding groove. This might be a consequence of the Val/Leu exchange at peptide position 9. The result is very interesting with respect to the C-terminal amino acids of a peptide that were found to take part in the interaction between HLA-Cw4 antigen and KIR2DL1 (Fan et al., 2001). However, to elucidate the influence of a shift observed in structures of pLMP2 peptide with some HLA-B27 subtypes on the binding of receptors present on the surface of the NK cells, the crystal structure of the HLA-B27 antigen complexed with pLMP2/pVIPR and the receptor should be provided.

4.3.5 Do the results presented in this study support currently discussed molecular mimicry hypothesis?

Comparison of all the mentioned structures allowed to establish whether the molecular mimicry found in B*2705 and B*2709 in complexes with pLMP2 and pVIPR (Fiorillo et al., 2005) can also be observed for B*2704 and B*2706 in complexes with the same peptides. The result was expected to lend support to the arthritogenic peptide hypothesis (Benjamin and Parham, 1990; Ramos and López de Castro, 2002). This hypothesis requires the self and viral peptides to feature extremely similar conformations when bound to the B*2705 subtype that could have consequences on the recognition of the peptide-bound antigen by the T cell receptor. I hoped to observe a similar situation in B*2704 subtype since position 116 of the HC is occupied by Asp

like in B*2705. From the peptide binding modes (all in p4α conformation) in the B*2704 and B*2706 subtypes, we could suspect that molecular mimicry holds in both cases. An additional surface representation analysis contradicts this conclusion, since each of the four analyzed structures looks different when seen from the top of the binging groove (Figure 4.4), however, the B*2706 does not reflect full peptide binding mode since p5 and p6 side chains of pVIPR could not be modeled due to disorder.

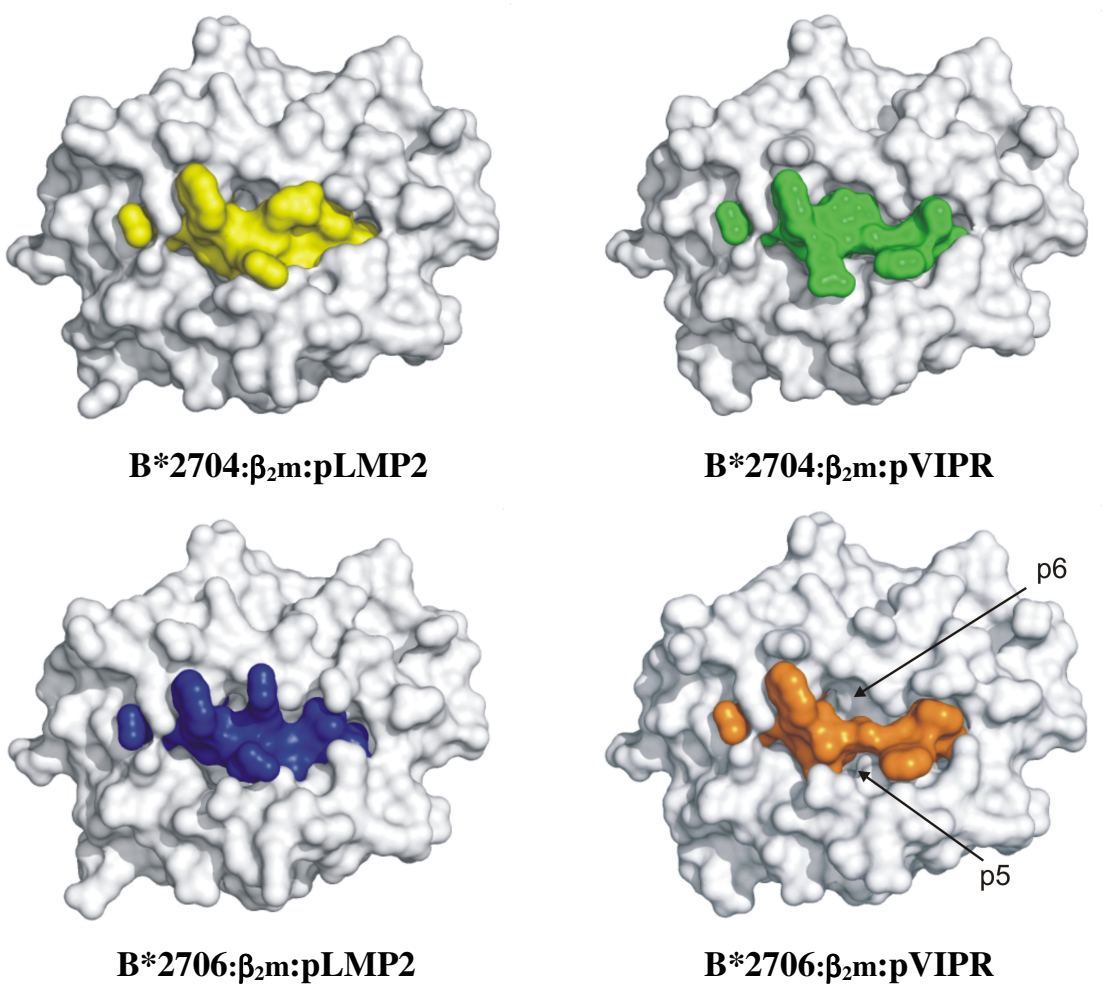

Figure 4.4. Molecular surface analysis of B*2704 and B*2706 in complexes with self and viral peptides. Structures of B*2704 and B*2706 with pLMP2 and pVIPR are shown in surface representation, the peptides are color-coded. p5 and p6 in B*2706:β_2m:pVIPR structure are not resolved, possible positions of the two amino acids are indicated by arrows.

Contrary to the finding for B*2705 and B*2709 antigens (Figure 1.6), the molecular mimicry in B*2704 and B*2706 in complexes with self and viral peptides is not that much pronounced. This can be explained by differences in side chains positions of peptide residues, since the backbone conformations are p4α in all four structures. The only difference in Cα atoms is found at position p6 of the pLMP2 and pVIPR backbones where the Cα–position of pLMP2 is

shifted by 2.0 Å toward the α1-helix (Figure 4.5). This is caused by pArg6 which in the self-peptide pVIPR contacts amino acids from the α1-helix, whereas pArg6 in pLMP2 is pointing toward the solvent. This difference at position 6 between pLMP2 and pVIPR seems to be characteristic for the p4α conformation and can be found also in this binding mode in B*2703, B*2705 and B*2709.

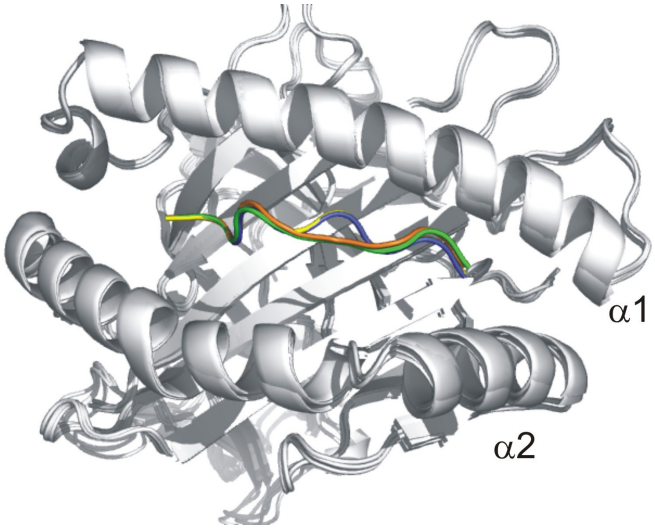

Figure 4.5. Overlay of B*2704 and B*2706 subtypes in complexes with pLMP2 and pVIPR. The structures are in ribbon representation. Peptides are color-coded. pLMP2 in B*2704 is yellow, in B*2706 – blue, pVIPR in B*2704 is green, in B*2706 – orange.

The experiments performed in this study and the presented results indicate that the finding about molecular mimicry can not be generalized. However, on the basis of the X-ray structures of B*2704 and B*2706 in complexes with self- and viral peptides we can conclude that the polymorphism of HLA subtypes in the binding groove might have an influence on peptide presentation as already shown for B*2705 and B*2709 (Fiorillo et al., 2005; Hülsmeyer et al., 2004).

4.3.6 Can expression and reconstitution systems for KIR3DL1 be developed?

Until now, only very few crystal structures for KIRs have been solved (Fan et al., 1997; Maenaka et al., 1999; Snyder et al., 1999; Boyington et al., 2000; Fan et al., 2001), and no structure for a 3-domain KIR such as KIR3DL1, which we investigated, is available. Two of the few structures that have been determined concern an HLA molecule bound to a 2-domain KIR: HLA-Cw3:KIR2DL2 (Boyington et al., 2000) and HLA-Cw4:KIR2DL1 (Fan et al., 2001).

It seems that the additional D0 domain of KIR3DL1, which is absent in the 2-domain KIRs, is a factor which interferes with protein production and/or crystallization. This domain is needed for the recognition of HLA-Bw4 molecules (Rojo et al., 1997; Khakoo et al., 2002), and appears to enhance the affinity for HLA (Khakoo et al., 2002). The KIR3DL1 binds only to HLA-B molecules with the Bw4 serological determinant.

Previous experiments with 2-domain KIR have shown that the protein can be expressed in *E.coli,* in the form of inclusion bodies (Snyder et al., 1999). As shown in section 3.4.2 the production of KIR3DL1 by this system yielded nearly pure protein. Experiments which were performed with KIR2DL2 (Snyder et al., 1999) and KIR2DL1 (Maenaka et al., 1999) indicate that it was possible to refold KIR alone, and the complex with its ligand was obtained by mixing both components in equal molar ratio. Obtaining KIR3DL1 in the refolded state would allow for complex preparation with HLA-B27 molecule as well as for its crystallization in a heterotetrameric form. In this study we tried to produce and refold the 3-domain KIR, however, there was no previous trial which indicated that a properly folded molecule could be obtained. All refolding and purification results described in section 3.4.3.1 show that the protein aggregates and it was not possible to obtain refolded KIR3DL1 from the aggregates.

The 3-domain KIR contains three immunoglobulin-like domains and three disulphide bridges. Whether this can be the cause for the molecular aggregation it is not known, however, two Ig-like domains of a 2-domain KIR could be refolded to protein. This suggest that it is possibly the D0 domain which gives rise to refolding problems of KIR3DL1, but its presence was found to be necessary for the binding to the Bw4 epitope (Rojo et al., 1997; Khakoo et al., 2002). In our experiments with KIR3DL1 *in vitro* refolding in presence of HLA-B*27 (B*2704 and B*2706), β_2m and peptide, the major peak after size exclusion purification contained aggregates. Testing different conditions of refolding time and refolding buffer composition, we were able to produce the complex only in small amount which allowed for SDS-PAGE analysis, but not crystallization. Previously described refolding conditions for proteins containing cysteins (Rudolph and Lilie 1996) were also not successful in case of KIR3DL1.

Very interesting results were presented for trehalose dihydrate (Singer and Lindquist 1998) which is known to be produced by a variety of organisms during stress and was found to stabilize proteins during heath shock. Even more interestingly, trehalose is a factor which suppresses the aggregation of proteins, maintaining them in a partially-folded state. However, trehalose as well as the recently released Stabil-PAC (Novexin) applied in our experiments with Killer Cell receptor did not help in complex production. The peptide pEBV8TvE used for complex formation is known to protect NK lysis (Stewart-Jones et al., 2005) which means that it could

enhance KIR binding to pMHC. We tested two other peptides with such properties (von Biuneudijk et al., 1992; Maluati et al., 1995) and additionally two which did not protect from NK lysis (Jardetsky et al., 1991; Maluati et al., 1995). Surprisingly there was no difference in complex formation between all the peptides used. The experiments performed in this study involve *in vitro* methods, and this may explain why the peptides which are known to prevent inhibitory KIR binding to pMHC *in vivo,* gave a complex of B*2705:β_2m:peptide-KIR3DL1 as well. This experiment provides evidence that the in vitro results could be misleading and have to be interpreted with caution.

Other receptors which inter alias are present in the subset of NK cells are LIR molecules (Chapman et al., 2000; Allen et al., 2001; Willcox et al., 2003). They are similar to KIR molecules as they contain two immunoglobulin-like domains in the extracellular part. Since they bind to the α3 domain of HLA and not to the top of the binding groove, it was expected that the LIR receptor might stabilize the HLA-KIR interaction and aid in complex formation. However, the aggregating properties of KIR3DL1 appeared to be far stronger than the stabilizing activity of any molecules and substances which were tested. This indicates that the protein itself has a very strong predisposition to aggregate and it seems to be difficult to produce it in refolded state either alone or in complex with pMHC in larger amounts.

However, the reconstitution of KIR3DL1 with B*2705:β_2m:pEBV8TvE from one of the many conditions which were tested produced sufficient amounts of the complex for crystallization set-ups. The produced complex contained all components and corresponded in size to the HLA molecule and KIR. Additionally, MALDI-TOF/TOF analysis confirmed the presence of the KIR3DL1 protein. Unfortunately, crystallization screens performed at 20°C as well as at 4°C did not give any crystals which had the proper size for harvesting and X-ray data collection.

From this study it can be concluded that the 3-domain KIR is a molecule which preferentially creates both aggregates of itself and aggregates of complexed components. This was demonstrated by time course experiments where aggregates were obtained already 4 hrs after KIR3DL1 was added to the reconstituted B*2705:β_2m:peptide complex. KIR aggregation mediated by bivalent metal cations in NK cell inhibition was previously described. It was demonstrated that KIR clustering on the cell surface follows recognition of protective HLA-C molecules on a target cell (Davis et al., 1999). It was also shown that soluble dimers of KIR2DL1 bind more strongly to HLA-C than the monomer (Fan et al., 2000 a; Fan et al., 2000 b). This is important in the light of the finding that the affinity of KIRs to MHC class I molecules is weak (about 10 μM; Vales-Gomez et al., 1998). Binding of two KIR molecules

through their D2 domains was suggested to bring together their cytoplasmic tails where the N-terminal ends would possibly be linked by bivalent cations (Fan et al., 2000b). Although crystallography revealed that KIR-KIR and HLA-C-KIR binding is different than expected (Snyder et al., 1999; Fan et al., 2001) and none of them resembles aggregates, the hypothesis about KIR aggregation can not be excluded since the crystallization was performed in the absence of bivalent cations (Vilches and Parham, 2002). The experiments described here demonstrate that both complex formation of HLA-B27 with 3-domain KIR as well as its crystallization are very difficult.

4.4 Importance of the presented results for HLA-B27 biology.

All experiments performed in this study were aimed to verify and confirm that HLA-B27 is a very important gene family associated with Ankylosing Spondylitis, although it has to be stated clearly that not all alleles are equally AS associated. The pathogenic role of HLA-B27 has been already established and the main properties of the molecule have been discussed (López de Castro 2007); however, different biological features of the molecule might cause diseases. The characteristics that provide evidence for the pathogenic role of HLA-B27 are: specificity of peptide presentation, possibility of misfolding, and formation of heavy chain homodimers. It has been postulated that the often occurring misfolding of HLA heavy chains might be a reason for the disease (Colbert; 2000). This could be an effect of misfolded molecules which accumulate in the ER, induce stress in the endoplasmic reticulum and disturb the normal physiological state in this compartment, leading to the activation of inflammatory pathways. Part of misfolded molecules in ER constitutes β_2m-free dimers which have also been identified on the cell surface which is caused by HLA-B27 overexpression (Antoniou et al., 2004). It was reported that subtypes which are non-AS-associated fold more efficiently than AS-associated ones (López de Castro, 2007), suggesting a correlation between disease association and misfolding of an HLA. However, recent results show equal misfolding ratio in two differently AS associated subtypes (B*2705 and B*2709) and suggest that different disease susceptibility cannot be linked to dimer formation and misfolding of the molecules (Giquel et al., 2007).

There might also be HLA homodimers which are formed on the cell surface, and these can be recognized by leukocyte receptors like KIR3DL1 (Allen et al., 2001 and Kollnberger et al., 2002). A hypothesis about the immunomodulatory effect of this recognition on diseases associated with HLA has been proposed (Allen and Trowsdale, 2004).

Spondyloarthropathy might also be caused by T-Cell autoimmunity against self-peptides of HLA-B27 initiated by cross-reactive antigens derived from pathogens (Benjamin and Parham, 1990; Hülsmeyer at al., 2004; Fiorillo et al., 2005, and Rückert et al., 2006). It is difficult to establish a set of arthritogenic peptides as well as explain why two AS associated subtypes (e.g. B*1403 and B*2705) share only 3% of their peptide repertoires (Merino et al., 2005). Since crossreactivity of T cells has been investigated with the pLMP2 and pVIPR peptides using two differently AS-associated subtypes, our experiments with the same peptides and with another pair of HLA-B27 subtypes, differing in AS association, shed light on currently discussed issues. Unexpectedly, the binding mode of pLMP2 and pVIPR peptides in B*2704 and B*2706 is similar in all cases and does not show such discrepancies as observed for B*2705 and B*2709. On the one hand, the result for B*2704 supports the arthritogenic peptide theory since this subtype is associated to AS, and the self peptide seems to mimic the viral peptide when bound to the groove, however, it is difficult to conclude whether the molecular mimicry is present in the light of results obtained for B*2706. Although the existence of molecular mimicry in B*2704 and B*2706 can not be stated unequivocally, the similarities in surface representations between self- and viral peptide are here definitely less pronounced as in B*2705 and B*2709.

Thanks to crystal structures which were solved in the course of the present study, a comparative analysis of several HLA-B27 subtypes became possible which allowed to establish that the self- and the viral peptide behave differently in numerous HLA subtypes. Various amino acid exchanges within the peptide binding groove did not result in the distinct pLMP2 peptide positions (B*2703, B*2704, B*2706), whereas the pVIPR peptide is presented differently by one and the same subtype B*2705. Nevertheless detailed analysis of each polymorphic residue located in the binding groove shows that Asp114 in B*2706 and Glu152 in B*2704 and B*2706 might explain the peptide binding mode or at least give a reason for residues positions.

To understand what happens when peptides are already attached, how strongly they are bound and what the interaction with the receptor looks like, a number of further experiments should be performed. Fluorescence polarization with a labeled peptide would help to understand movements of the peptides within the binding groove. To establish the basis for HLA-B27 recognition by the receptors, the structure of HLA-B*2703/04/06 with peptide and TCR or NK should be provided and analyzed in detail. The recognition pattern of an HLA-B27 subtype by the KIR receptor was one of the subjects under investigation in the present study. To provide a structure of KIR3DL1 bound to an HLA-B27 subtype several methods of protein production and purification were tried (e.g. bacteria and yeast system as well as S2 cells for protein production, affinity chromatography). Only the most successful techniques, which allowed to produce

complex of HLA-B27 with KIR3DL1 in a quality and quantity sufficient for crystallization, were described here. However, the structure of a three domains KIR with an HLA-B27 subtype could not be solved so far.

The presence of AS among people from different populations bearing different HLA-B27 alleles confirms the importance of this gene family in AS pathogenesis. An approach must be taken to learn more about the biological implications as well as about the efficient treatment of AS.

SUMMARY

Autoimmune diseases are characterized by cellular or antibody responses against self-antigens. However, the precise role of antigens specified by the major histocompatibility complex (MHC) in these diseases, although crucial, has not been determined. In case of the human MHC gene *HLA-B27* and its association with the rheumatoid disease Ankylosing Spondylitis (AS), the HLA-B27 protein itself is the strongest predisposing factor for pathogenesis. It is possible that the molecule binds self- and viral peptides in an extremely similar way (molecular mimicry) and disables distinguishing of peptides by T cells. The *HLA-B*2706* subtype exhibits no association to AS and its product differs only at two amino acid positions (114 and 116) in the peptide binding groove from that of the AS-associated subtype *HLA-B*2704*. Similarly, the *HLA-B*2703* subtype, which exhibits a questionable AS-association, differs from the ancestral, AS-associated allele *HLA-B*2705* only in one residue. It is known that the self-peptide pVIPR (RRKWRRWHL) and the viral peptide pLMP2 (RRRWRRLTV) are presented in drastically different conformations by the subtypes HLA-B*2705 and HLA-B*2709 that differ only by an Asp116His exchange. The similar binding modes lead to molecular mimicry between the two peptides only when displayed by the AS-associated subtype HLA-B*2705. It is of great importance to establish whether this finding can be generalized.

Therefore, a structural analysis of the HLA-B*2704/B*2706 pair of subtypes as well as a comparison of the HLA-B*2703 and HLA-B*2705 subtypes, all complexed with pLMP2 and pVIPR peptides, may be expected to shed light on this problem. The results presented here show that molecular mimicry does not occur in the B*2704 subtypes probably due to very flexible side chains of the peptide amino acids. In B*2703 subtype both peptides have clearly defined distinct binding modes, and this excludes molecular mimicry. Furthermore, the binding of interacting proteins on effector cells including killer Ig-like receptors (KIR3DL1 in case of HLA-B27) may also be influenced, and this is a further topic of the thesis. Using X-ray crystallography, we attempted to solve the structure of KIR3DL1 bound to at least one of the HLA-B27:β_2m:peptide complexes whose structures had been solved previously. I produced the KIR3DL1 protein in the form of inclusion bodies in *E. coli* and tested a vast number of different conditions of reconstitution. The best complex was obtained for HLA-B2705 subtype with the pEBV8TvE peptide and KIR3DL1. The only microcrystal which was obtained in a crystallization process was too small for harvesting and X-ray data collection.

Results presented in this study show that even a close similarity between two HLA-B27 subtypes does not allow to predict how a given peptide is bound. Additionally, it is very difficult

to establish the basis for the recognition of an HLA-B27:β_2m:peptide complex by KIR3DL1, and further structural analyses still must still be performed in order to understand this important interaction.

ZUSAMMENFASSUNG

Autoimmunerkrankungen zeichnen sich durch zelluläre oder Antikörper Reaktionen gegen körpereigene Antigene aus. Jedoch die präzise Funktion der Antigene, die spezifisch für Haupt-Histokompatibilitätskomplexe (MHC) in diesen Erkrankungen sind, konnte trotzt ihrer wichtigen Rolle, noch nicht geklärt werden. Wenn es sich um die Gene *HLA-B27* von menschlichen MHC und ihre Assoziation mit der Ankylosing Spondylitis (AS) handelt, bilden die Proteine einen sehr starken Veranlagungsfaktor für die Pathogenese. Es ist möglich, dass die Eigen- und Viralpeptide von MHC in sehr ähnlicher Weise gebunden werden (molekulare Mimikry), was T-Zellen eine Unterscheidung der präsentierten Peptide unmöglich macht. Der Subtyp *HLA-B*2706* ist nicht AS assoziiert und seine Aminosäuresequenz unterscheidet sich von *HLA-B*2704,* der AS assoziiert ist, in zwei Aminosäuren (114 und 116), die sich in der Bindungsstelle des Peptides befinden. In ähnlicher Weise unterscheidet sich der Subtyp *HLA-B*2703*, vom ursprünglichen *B*2705* Subtyp, der AS-assoziiert ist, nur in einer Aminosäure. Es konnte jedoch nicht abschließend gezeigt werden, dass der Subtyp *B*2703* AS-assoziiert ist. Es ist bekannt, dass das körpereigene Peptid pVIPR (RRKWRRWHL) und das virale Peptid pLMP2 (RRRWRRLTV) von den Subtypen B*2705 und B*2709, die sich nur durch einen Asparagin zu Histidin Austausch an Position 116 unterscheiden, in äußerst unterschiedlichen Konformationen präsentiert wird. Die ähnlichen Bindungsmodi führen zur molekulare Mimikry zwischen zwei Peptiden, nur wenn sie vom AS-assoziiertem Subtyp HLA-B*2705 präsentiert werden. Es ist außerordentlich wichtig herauszufinden, ob diese Beobachtung verallgemeinert werden kann.

Deshalb ist eine strukturelle Analyse der B*2704 und B*2706 Subtypen und der Vergleich von B*2703 mit dem B*2705 Subtyp, wenn sie mit den pLMP2 und pVIPR beladen sind, nötig, um Licht auf diese Fragestellung zu werfen. Die hier präsentierten Ergebnisse zeigen, dass molekulare Mimikry im Subtyp B*2704 auf Grund der hohen Flexibilität der Aminosäure Seitenketten des Peptides wahrscheinlich nicht vorliegt. Im Subtyp B*2703 besitzen beide Peptide sehr unterschiedliche Bindungsmodi, und somit kann molekulare Mimikry ausgeschlossen werden. Darüber hinaus könnte die Bindung interagierenden Proteinen auf Effektorzellen, eingeschlossen dem Ig-ähnlichen Killer-Rezeptor (KIR3DL1 im Falle von HLA-B27) beeinflusst werden und dies ist ein weiterer Punkt dieser Arbeit. Mit Hilfe der Röntgenstrukturanalyse versuchten wir die Struktur eines KIR3DL1 welcher an einen HLA-B27:Peptid Komplex gebunden ist, dessen Struktur bereits bekannt ist, zu ermitteln. Ich habe das KIR3DL1 Protein in Form von Einschlusskörpern in *E. coli* produziert. Es wurde eine

Vielzahl verschiedener Rekonstitutionsbedingungen getestet. Der beste Komplex wurde für den Subtyp B*2705, der mit dem Peptid pEBV8TvE und KIR3DL1 beladen ist, erzielt. Der einzige Mikrokristall der durch Kristallisation erhalten wurde, war allerdings zu klein, um geerntet zu werden und um kristallgraphische Daten zu sammeln.

Die dargestellten Forschungsergebnisse deuten darauf hin, dass die Ähnlichkeit zwischen zwei HLA-B27 Subtypen nicht erlaubt vorherzusagen, wie das Peptid gebunden ist. Darüber hinaus, es ist sehr schwer die Grundlage der Interaktion zwischen einem HLA-B27:Peptid Komplex und KIR3DL1 zu bestimmen. Weitere Strukturanalysen müssen unternommen werden, um diesen wichtigen Prozess zu verstehen.

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LIST OF PUBLICATIONS

1. Loll, B., Zawacka, A., Biesiadka, J., Petter, C., Rückert, C., Saenger, W., Uchanska-Ziegler, B., Ziegler, A. (2005) Preliminary X-ray diffraction analysis of crystal from the recombinantly expressed human major histocompatibility antigen HLA-B*2704 in complex with a viral peptide and a self-peptide. *Acta Crystallogr. Sect. F Struct. Cryst. Commun.* 61, 939-941.

2. Loll, B., Zawacka, A., Biesiadka, J., Ruckert, C., Volz, A., Saenger, W., Uchanska-Ziegler, B., Ziegler, A. (2005) Purification, crystallization and preliminary X-ray diffraction analysis of the human major histocompatibility antigen HLA-B*2703 complexed with a viral peptide and with a self-peptide. *Acta Crystallogr. Sect. F Struct. Cryst. Commun.* 61, 372-374.

3. Zawacka, A., Loll, B., Biesiadka, J., Saenger, W., Uchanska-Ziegler, B., Ziegler, A. (2005) X-ray diffraction analysis of crystals from the human histocompatibility antigen HLA-B*2706 in complex with a viral peptide and with a self-peptide. *Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun.* 61, 1097-1099

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