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PEPTIDE MOTIF REGISTER

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The role of the amino acids associated with the HLA-Bw4/Bw6 epitope in peptide binding to HLA-B5, B35 CREG molecules

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HLA-B51, HLA-B53, and HLA-B35 molecules form part of a serologically cross-reactive group (CREG) called HLA-B5, B35 CREG which includes HLA-B52 and HLA-B78. HLA-B*5301 is a hybrid molecule in which the α1 domain of HLA-B*3501 has been replaced with that from HLA-B*5101. HLA-B*5301 differs from HLA-B*3501 by only five amino acids (residues 77, 80, 81, 82, and 83) which are associated with the Bw4/Bw6 epitope. Residues 77, 80, and 81 form the F-pocket (Smith et al. 1996a, b). Pool sequencing studies of self-peptides eluted from HLA-B*3501 and HLA-B*5101 molecules showed that these peptides have either Pro or Ala at position 2 (P2) and Ile, Leu or Met at position 9 (P9), and in addition that HLA-B*3501, but not HLA-B*5101, favors peptides carrying Tyr at the C-terminus (Falk et al. 1993, 1995). These findings were confirmed by peptide binding studies using chemically synthesized peptides (Sakaguchi et al. 1997; Schönbach et al. 1995, 1996). Pool sequencing studies of both naturally processed self-peptides eluted from HLA-B*5301 molecules (Hill et al. 1992) and of peptides eluted from HLA-B*5301 refolded with randomly synthesized nonamer peptides (Davenport et al 1997) showed that Pro was also a P2 anchor for HLA-B*5301. These studies further implied that there is no amino acid preference in the C-terminus of B*5301 binding peptides.

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To investigate whether HLA-B*5301 binding peptides have hydrophobic residues in the C-terminal anchor, we tested the binding of 62 nonamer peptides with Pro at P2, and Phe, Ile, Tyr, Leu, or Met at P9, which are known to be the C-terminal anchors for HLA-B*3501 or HLA-B*5101 (Sakaguchi et al. 1997; Schönbach et al 1995). Peptide binding to HLA-B*5301 was measured by the stabilization assay (Schönbach et al 1995) using RMA-S-B*5301 cells (Nakayama et al. 1994). The percentage of HLA-B*5301 binding peptides with Phe, Ile, Tyr, Leu, and Met at P9 was 85.7%, 58.8%, 58.3%, 58.8%, and 11.1%, respectively. The MBR was highest for peptides with Phe at P9 and lowest for peptides with Met at P9 (Table 1). These results suggest that Phe, Ile, Tyr, and Leu are all C-terminal anchor residues for HLA-B*5301. This was confirmed by determining the affinity of peptides whose C-terminal residues had been substituted (Fig. 1). Peptides with Phe at the C-terminus had the highest affinity to HLA-B*5301 molecules, with the binding affinity reduced by, in order, Ile, Tyr, Leu, and Met, being at the C-terminus.

Although both HLA-B*3501 and HLA-B*5301 binding peptides have similar C-terminal anchor residues, there is a C-terminus preference: Tyr is favored in HLA-B*3501 binding peptides, while Phe is favored in B*5301 binding peptides. This difference can be explained by the crystal structures of both HLA-B*5301 complexed with KPIVQYDNF or TPYDINQML and HLA-B*3501 complexed with VPLRPMTY (Smith et al. 1996a, b). A Tyr hydroxyl group at the C-terminus of the binding peptide can form a hydrogen bond with Ser at residue 116 and Tyr at residue 74. In HLA-B*3501 the relatively small Ser at residue 77 allows a hydrogen bond to be made via a water molecule to the P6 carbonyl group while increasing the available volume of the F pocket. The Tyr specificity of the F-pocket of HLA-B*3501 arises from the ability of the Tyr hydroxyl group to hydrogen bond to both Ser at residue 116 and Tyr at residue 74, and this is attributable to the polymorphic residues 77, 80, and 81. In contrast, the

Table 1 Effect of C-terminus anchor on binding of nonamer peptide to HLA-B*5301 molecules

	Amino acid of the C-terminus					
	F	I	Y	L	M	Total
N.B.P.[1]/N.T.P.[2]	6/7	10/17	7/12	10/17	1/9	34/62
(percentage)	(85.7%)	(58.8%)	(58.3%)	(58.8%)	(11.1%)	(54.8%)
MBR[3]	1.43	1.18	1.00	0.94	0.22	0.97

[1] Number of binding peptides. Binding peptides were evaluated as follows: Briefly, RMA-S-B*5301 cells (Nakayama et al. 1994) were cultured at 26 °C for 18 h. Cells (2×10^5) in 50 μl of phosphate buffered saline (PBS) supplemented with 20% FCS (PBS-FCS) were incubated at 26 °C for 3 h with 50 μl of a solution of peptides at $10^{-3} \sim 10^{-7}$ M and then at 37 °C for 3 h. After washing with PBS-FCS, cells were incubated for 30 min on ice with an appropriate dilution of TP25.99 anti-HLA class I α3 domain monoclonal antibody. After two washes with PBS-FCS, cells were incubated for 30 min on ice with an appropriate dilution of fluorescein isothiocyanate-conjugated IgG of sheep mouse-specific Ig antibodies (Silenus Laboratories, Hawthorn, Australia). Cells were then washed three times with PBS-FCS and the fluorescein intensity was measured using a FACScan flow cytometer (Becton Dickinson, Mountain View, Calif.). The percentage binding affinity was calculated as follows. Mean fluorescence intensity (MFI) at the peptide concentration of 10^{-3} M minus the background MFI was divided by maximal MFI minus the background MFI. Peptides giving more than 25% binding affinity were evaluated as binding peptides. The half maximal binding level (BL_{50}) which is defined as the peptide concentration yielding the half-maximal MFI was calculated

[2] Number of tested peptides

[3] The mean binding rank. MBR was calculated as follows: binding peptides were classified according to the BL_{50} into three categories: high binder ($BL_{50} < 10^{-4}$ M), medium binder ($10^{-4} < BL_{50} \leq 10^{-3}$) and low binder ($BL_{50} > 10^{-3}$). High, medium, low, and nonbinder were then given the ranks 3, 2, 1, and 0, respectively. Average of the rank of peptides was shown as MBR

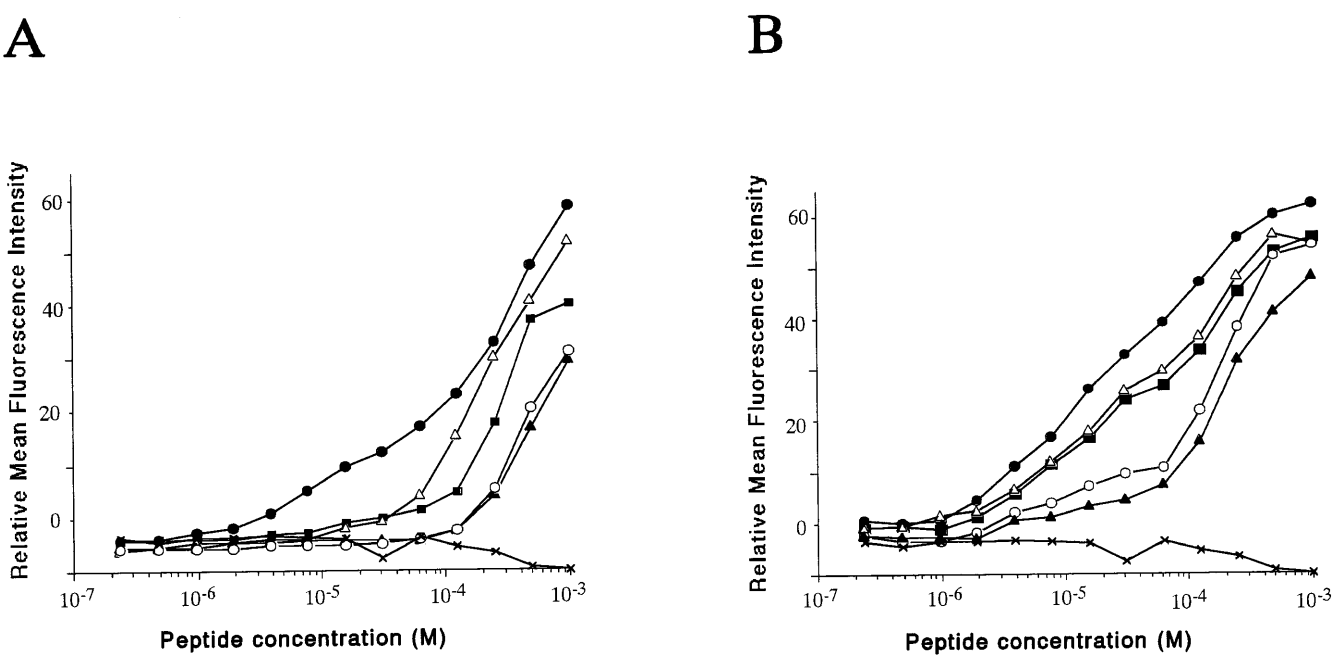

Fig. 1A, B Effect of C-terminal anchor residues on peptide binding to HLA-B*5301 molecules. The binding affinity of mutated peptides to HLA-B*5301 molecules was measured by a stabilization assay using RMA-S-B*5301 cells. The C-terminus of peptides **A** NPDIVIYQY and **B** IPLTEEAEL was replaced by Phe (*closed circle*), Ile (*open triangle*), Tyr (*closed square*), Leu (*open circle*), or Met (*closed triangle*). The peptide CQLDPARDV(X) was used as a negative control

Ala at residue 81 of HLA-B*5301 allows a C-terminus Phe to fill the F-pocket and pack against Tyr at residue 123 and Asn at residue 77.

We have previously shown that the affinity of peptides to HLA-B*5101 molecules is much weaker than to HLA-B*3501 molecules (Sakaguchi et al. 1997). This implies that the structure of the F-pocket may determine peptide affinity in these HLA class I molecules. However, since HLA-B*5101 and HLA-B*3501 molecules differ structurally in other pockets as well, these other differences could also influence the peptide affinity . As HLA-B*5301 and HLA-B*3501 differ by only five amino acids associated with the Bw4/Bw6 epitopes forming part of the F-pocket, a comparison of both HLA-B*5301 and HLA-B*3501 binding peptides could address this question.

We investigated the affinity of epitope peptides presented by these three HLA class I molecules. Only two HLA-B53-restricted CTL epitopes have been reported, one derived from HIV-2 (TPYDINQML) (Gotch et al. 1993) and one from *Plasmodium falciparum* (KPIVQYDNF) (Hill et al. 1992). The affinity (BL_{50}) of these

Table 2 Affinity of CTL epitope peptides to HLA class I molecules

HLA-B*5301-restricted epitopes	BL_{50}[1]	HLA-B*3501-restricted epitopes	BL_{50}	HLA-B*5101-restricted epitopes[2]	BL_{50}
T P Y D I N Q M L	2.4×10^{-5}	E P I V G A E T F	7.4×10^{-8}	L P P V V A K E I	6.1×10^{-6}
K P I V Q Y D N F	2.9×10^{-5}	F P V R P Q V P L	1.2×10^{-7}	T A F T I P S I	6.8×10^{-6}
		I P L T E E A E L	1.4×10^{-7}	D A Y F S V P L	2.3×10^{-5}
		N P D I V I Y Q Y	3.7×10^{-7}	L P C R I K Q I I	7.3×10^{-5}
		D P N P Q E V V L	7.5×10^{-6}	N A N P D C K T I	4.0×10^{-4}
		E P I V G A E T F Y	1.2×10^{-5}	Q G W K G S P A I	$>10^{-3}$
		R P Q V P L R P M T Y	2.7×10^{-5}		
		V P L D K D F R K Y	4.5×10^{-5}		
		R P I V S T Q L L	1.1×10^{-4}		

[1] BL_{50} was defined as described in the Table 1 legend
[2] BL_{50} of HLA-B*5101-restricted epitopes has been previously shown (Tomiyama et al. 1999)

Table 3 Binding of peptides carrying Pro at P2 and Phe, Ile, Tyr, Leu, and Met at the C-terminus to HLA-B*5301 and HLA-B*3501 molecules

Nonamer peptides	BL_{50}	
	HLA-B*5301	HLA-B*3501
Peptides carrying Phe at P9		
L P K L P G V P F	$>10^{-3}$	7.4×10^{-7}
A P P E E S F R F	$>10^{-3}$	1.1×10^{-5}
S P P A V P Q T F	$>10^{-3}$	3.4×10^{-5}
D P E I V M H S F	$>10^{-3}$	2.9×10^{-4}
P P H S A K S K F	NB	NB
Peptides carrying Ile at P9		
Y P C T V N F T I	1.4×10^{-5}	3.9×10^{-6}
L P C R I K Q I I	1.6×10^{-4}	6.6×10^{-6}
L P P T T G P P I	$>10^{-3}$	2.2×10^{-4}
Q P R G R R Q P I	NB	NB
Peptides carrying Tyr at P9		
S P S S T P L L Y	1.5×10^{-5}	4.4×10^{-7}
L P I V G A E T Y	2.0×10^{-4}	5.2×10^{-7}
V P G A A Y A L Y	$>10^{-3}$	1.1×10^{-4}
G P K E P F R D Y	NB	NB
Peptides carrying Leu at P9		
T P I P A A S Q L	3.8×10^{-5}	2.3×10^{-6}
W P L Y G N E G L	1.1×10^{-4}	3.1×10^{-5}
Y P L T S L R S L	$>10^{-3}$	3.5×10^{-6}
P P A V H G C P L	NB	$>10^{-3}$
Peptides carrying Met at P9		
R P I V S T Q L M	1.5×10^{-4}	3.2×10^{-5}
A P P P S W D Q M	NB	7.5×10^{-5}

peptides was 2.4×10^{-5} and 2.9×10^{-5}, respectively (Table 2). Nine HLA-B*3501-restricted CTL epitopes were tested by the same experimental protocol with RMA-S-B*3501 cells. The BL 50 of these epitopes was 1.1×10^{-4} to 7.4×10^{-8} (Table 2). In a recent study using the same experimental protocol, we showed that the BL_{50} of six HLA-B*5101-restricted epitope peptides was 6.1×10^{-6} to $>10^{-3}$ (Table 2) (Tomiyama et al. 1999). Thus, the affinity of HLA-B*5301-restricted epitopes is similar to that of HLA-B*5101-restricted epitopes, but is much lower than that of HLA-B*3501-restricted epitopes. These observations suggest that the Bw4/Bw6 epitopes determine peptide affinity to HLA-B5, B35 CREG molecules.

In order to directly demonstrate a role of the Bw4/Bw6 epitopes in peptide binding, we examined the binding of HLA-B*5301 binding peptides to HLA-B*3501 molecules. Fourteen HLA-B*5301 binding peptides and five HLA-B*5301 nonbinding peptides were tested using RMA-S-B*3501 cells. While three HLA-B*5301 nonbinding peptides also did not bind to HLA-B*3501, the affinity of the remaining peptides to HLA-B*3501 was much higher than to HLA-B*5301 (Table 3). This directly demonstrates that the Bw4/Bw6 epitope has a critical influence on peptide binding to these HLA-B molecules. The molecular mechanism of the role of the Bw4/Bw6 epitopes in peptide affinity for HLA-B*3501 and HLA-B*5301 is still unclear. Of the five amino acid differences between HLA-B*3501 and HLA-B*5301, only residues 77, 80, and 81 contribute to binding the C-terminus residue (Smith et al. 1996a, b). In HLA-B*3501, Asn at residue 80 can form a hydrogen bond with the C-terminal carboxyl group. This adds an additional hydrogen bond to the conserved hydrogen bonding network of the F-pocket. Leu at residue 81 of HLA-B*3501 fills the end of F-pocket, while the small Ala side chain of HLA-B*5301 increases the volume of the F-pocket. Therefore, these residues may influence the affinity of peptides for these HLA class I molecules, either satirically or electrostatically.

Apart from the five residues associated with the Bw4/Bw6 epitopes, HLA-B*5101 and HLA-B*3501 differ by eight residues in the $\alpha2$ domain. Since amino acid substitution at residue 116 (Tyr in HLA-B*5101 and Ser in HLA-B*3501) facing the F-pocket is thought to influence interactions with the C-terminus of peptides, this residue may play a critical role in peptide affinity. However, our recent study using an HLA-B*3501 mutant with Tyr at residue 116 showed that this substitution determines preference of the C-terminal amino acid but does not influence peptide affinity (Kubo et al. 1998). Though it remains possible that the

other residues on the $\alpha 2$ domain could influence peptide affinity, it is likely that the Bw4 epitope is responsible for the low affinity of HLA-B*5101 binding peptide.

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