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PEPTIDE MOTIF REGISTER

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Peptide binding motifs of the MHC class I molecules (RT1.A^l) of the Lewis rat

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Peptide binding to major histocompatibility complex (MHC) class I molecules is governed by allele-specific motifs composed of several conserved anchor positions (reviewed in Rammensee et al. 1995). The peptide binding motifs of the rat MHC class I molecules, RT1.A, have not been well characterized. Thus, except for one preliminary communication (Powis et al. 1993) and a recent report of the RT1.A^a motif (Powis et al. 1996), no other information on peptide specificity of RT1.A molecules has been published. The study of RT1.A motifs is interesting in view of the allelic dimorphism of the rat TAP transporter, which results in different sets of peptides being transported for loading onto RT1.A molecules of different haplotypes (reviewed in Howard 1995). Since the Lewis rat (RT1^l) is widely used in the study of transplantation and experimental autoimmune diseases, we sought to characterize a peptide binding motif of RT1.A^l using pool and individual sequencing of RT1.A^l-bound peptides.

In addition, peptide presentation by MHC class I molecules of different cell types has not been studied in detail. In this regard, T cells draw particular attention since MHC class I-restricted interactions between T cells were proposed to play a role in immune regulation (Sun et al. 1988; Zhang et al. 1993; Kuhrober et al. 1994). Therefore, it was of interest to analyze peptides presented by MHC class I molecules on T cells.

To this end, we isolated and sequenced peptides naturally bound to MHC class I molecules of the Lewis rat CD4$^+$, TCR$\alpha\beta^+$ T cell clone A2b (Holoshitz et al. 1984). During prolonged culture in vitro, a subclone of A2b acquired the ability to continuously grow in the presence of IL-2 without the addition of antigen-presenting cells, while maintaining its antigen specificity and phenotypic markers. The A2b cells were grown in Dulbecco's modified Eagle medium supplemented with 5% fetal calf serum and rIL-2, pelleted, and detergent-lysed. The lysate was precleared and passed over a column of RT1.A-specific mAb OX18 coupled to Sepharose. Although OX18 was reported to cross-react with non-classical rat class I products (Jameson et al. 1992), these molecules are usually poorly expressed. Indeed, OX18 and another RT1.A-specific mAb, F16-4.4., manifested equally strong staining of A2b cells, suggesting that RT1.A is the major, if not the only, class I protein expressed on these cells. The material bound to the affinity column was eluted with TFA and passed through Centriprep 3 ultrafiltration membranes (Amicon, Beverly, MA). The filtrate was concentrated and separated using reverse-phase HPLC as described (Falk et al. 1994). Dominant single peaks were sequenced individually, whereby the remaining fractions were pooled and sequenced as such. The fractions were sequenced by Edman degradation on a pulsed-liquid protein sequencer 476A with on-line PTH-a.a. analysis (Applied Biosystems Weiterstadt, Germany). The pool sequencing data were interpreted as described (Falk et al. 1991).

The results of peptide sequencing are summarized in Table 1. Pool sequencing of RT1.1A^l-bound peptides showed two major anchor positions, 3 and 9, occupied by aromatic Phe and Tyr residues. Aliphatic residues such as Leu and Met also contribute to the P9 anchor. An auxiliary anchor was observed at position 2, showing strong signals for small residues such as Ala, Ser, and Val. Minor preferences were detected at other positions, such as small or negatively charged residues at positions 7 and 8. A detailed listing of preferred and other detected amino acids is given in Table 1. In addition, eight individual peptides were sequenced, six of them matching known proteins. All these peptides contain the proposed RT1.A^l motif. Although it cannot be formally ruled out that some detected residues could be contributed by non-classical

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Table 1 Sequencing of RT1.A^{l}-eluted peptides

A Pool sequencing

Position	1	2	3	4	5	6	7	8	9
Anchors or auxiliary anchors		A S V	**F Y**						**Y F L M**
Preferred	A S K		L	E L K P G Q	E D F	R K	E D	E T D	
Also present	G Y F V			D V	A S	I L V	A S T V	A S	

B Individual ligands

Fr.	Sequence	Source	Position	Accession
17	GG**Y**GGGYDN**Y**	hnRNP A2 (human)	281–290	P22626
21	AG**Y**GGYEE**Y**	hnRNP F (human)	238–246	L28010
26	GS**Y**NDFGN**Y**	hnRNP A1	257–265	P04256
33	SA**F**RNVTS**F**	Unknown		
35	NA**F**KEITT**M**	TFIIB	116–124	P29053
39	AA**F**FNKTE**F**	Unknown		
49	MSLIINTF**Y**	HSP90 beta	24– 32	P34058
54	NI**F**KFIIP	LDH M chain	115–122	P04642

class I molecules, the emergence of a single prominent motif in both individual and pool sequencing suggests its origin from RT1.A.

The RT1.A^{l} peptide binding motif reported here is in keeping with a preliminary report of a possible RT1.A^{l} motif based on pool sequencing (Powis et al. 1993). Like other class I molecules, RT1.A^{l} appears to bind short peptides of uniform length (9 amino acids) with a prominent C-terminal anchor position as well as two additional anchor positions. The specificity of RT1.A^{l} is consistent with the specificity of the corresponding *TAP* allele *TAP2A* (cim^{a}). Indeed, *TAP2A* can efficiently transport peptides with aromatic as well as other residues at the C-terminus (Heemels et al. 1993) and shows a strict preference for short (9 amino acids and shorter peptides (Heemels et al. 1994).

The sequencing of major peptide fractions presented by MHC class I molecules on T cells revealed that these peptides are derived from ubiquitously expressed nuclear and cytoplasmic proteins. These data suggest that the MHC class I presentation pathway in T cells acts along the lines of the other cell types studied. Similarly, we found that the peptides bound to MHC class II molecules of the same T-cell clone were derived from ubiquitous proteins (Reizis et al. 1996). Therefore, it appears that at least the most abundant peptides presented by MHC molecules on various cell types may not be tissue-specific. Further experiments are required to identify any T cell-specific peptides which might be involved in regulatory interactions between T cells. Nevertheless, the described RT1.A^{l} binding motif should be useful for predicting potential CTL epitopes for studies on transplantation, autoimmunity, and immunoregulation in the Lewis rat.

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