Identification of Novel B*57:01 Restricted Peptides from HIV gp140 using Immunopeptidomics

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BACKGROUND

- Elite controllers (EC) or Long Term non-progressors (LTNP)
  - Low viremia
  - Normal CD4+ T cell counts
  - Possess HLA alleles like B*57:01 or B*27:05
- The majority of the HIV epitopes described to date have been identified using synthetic peptide libraries
  - may fail to identify post-translationally modified epitopes, epitopes of non-canonical lengths
- Immunopeptidomics technology
  - Liquid chromatography-Mass Spectrometry (LC-MS) based complementary technique to overcome these limitations
- Subtle changes in peptides can have significant impact on T-cell recognition making it critical that these peptides are studied in their natural context.

Immune Surveillance: T Cells and MHC complexes

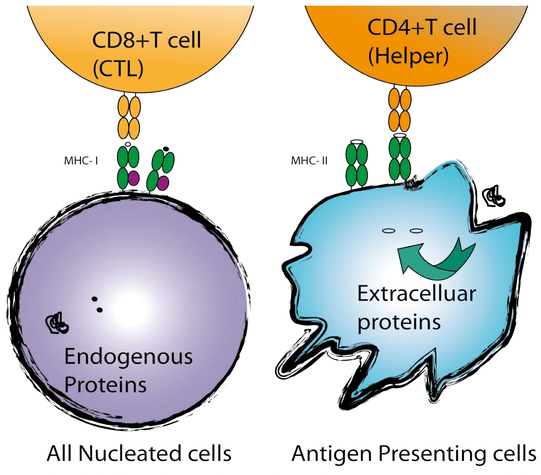


Figure 1: MHC class I molecules present peptides of endogenous origin while MHC class II molecules present proteins of extracellular origin.

Cell Lines: Generation of stable transfectants

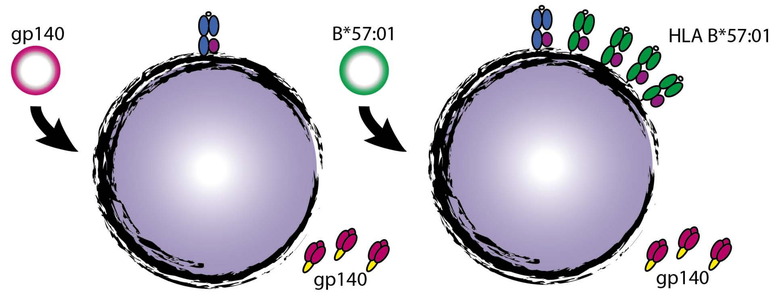


Figure 2: Preparation of cells. C1R cells were transfected initially with a gp140 plasmid (G418) followed by selection of stably transfected clones. C1R cells were further transfected with HLA B*57:01 (Hygromycin). Clones expressing both HLA B*57:01 and gp140 were chosen for expansion.

Immunoaffinity purification and Mass Spectrometry

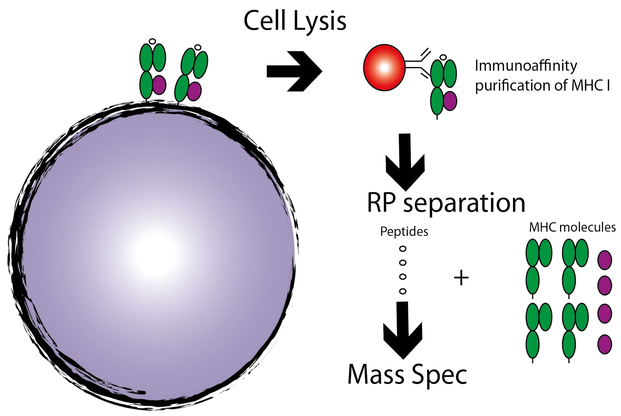


Figure 3: Overview of the MHC peptide elution and subsequent analysis by mass spectrometry.

RESULTS

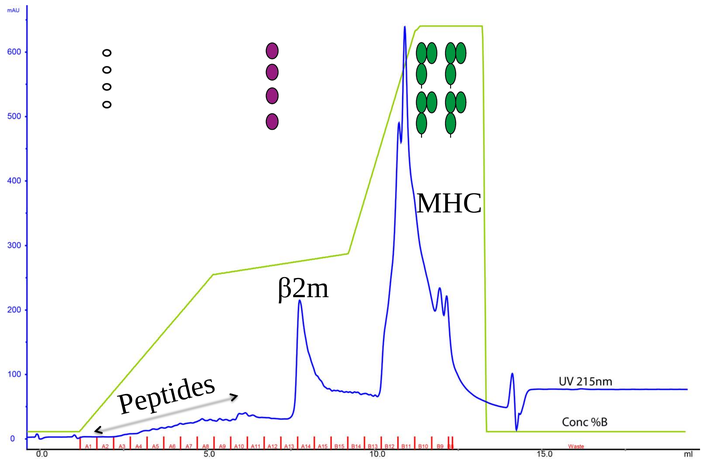


Figure 4: Peptides are separated from MHC molecules using reversed phase chromatography

B*57:01 ligands demonstrate anticipated distribution

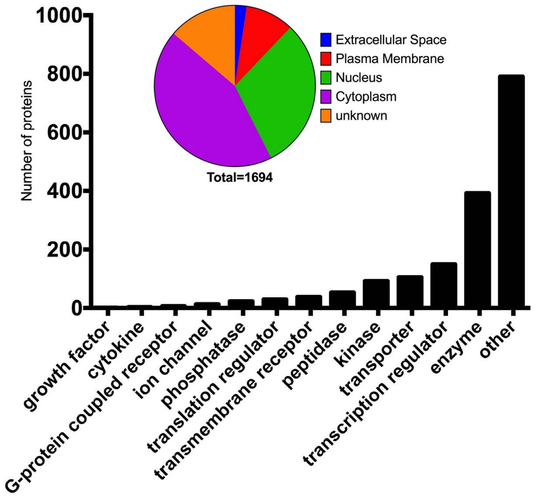


Figure 5: Functional classification of proteins represented by the B*57:01 bound peptides and their Cellular localization (inset)

9-, 10- and 11-mers predominate HLA repertoire

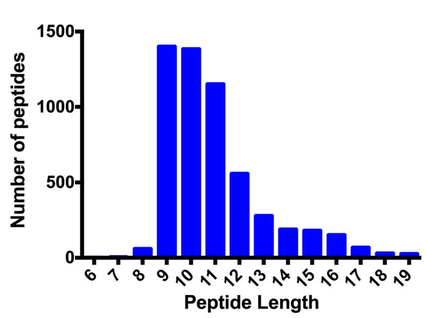


Figure 6: Frequency of distribution of length of B*57:01 peptides

Naturally processed HIV peptides identified

Sequence	Modifications	ΔMass	Time	Prec MW
Env				
RVKEKYQHLW		0.0021	10.62	1385.7589
RVKEKYQHLW	Trp->Kynurenin(W)@10	0.0044	10.221	1389.7561
RVKEKYQHLWRW		0.0036	13.441	1727.9409
KAYDTEVHNVW		0.0015	13.027	1360.6427
RIKQIINMW		0.0025	21.552	1200.6826
KSLEQIWNNMTW	Deamidated(N)@9	0.0078	44.406	1549.7313
KSLEQIWNNMTW		0.0089	40.289	1548.7484
Rev				
RTVRLIKLLY		0.0046	15.40	1273.8280

CONCLUSIONS

- First evidence of naturally presented kynurenine-modified peptide
- Several unexpected modified peptides were identified.
- Could be compared with alleles involved in progression (B8 and B7)
- T cell functional assays need to be performed to evaluate responses in LTNPs and progressors.

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