

Identification of antigenic peptides utilizing dedicated metabolic labeling strategies

Part II: MHC class II-associated peptides

Introduction

Professional antigen presenting cells are capable of initiating CD4⁺ T cell responses by displaying foreign peptides in the context of MHC class II molecules. Identification of these peptides results in a better understanding of intracellular antigen processing and is essential to study the immunogenicity of antigens, necessary for the development of improved vaccines. To facilitate the unambiguous distinction between the few pathogen-originating epitopes from the tens of thousands of self-peptides, we developed a dedicated labeling strategy adapted to the MHC class II processing pathway (figure 1). The strategy is based on the uniform metabolic ¹⁵N-labeling of the foreign pathogen and has been applied to unravel the epitope display of pathogen-originating peptides after pulsing dendritic cells with outer membrane vesicles or whole cells derived from *Neisseria meningitidis* serogroup B or from *Bordetella pertussis*, respectively.

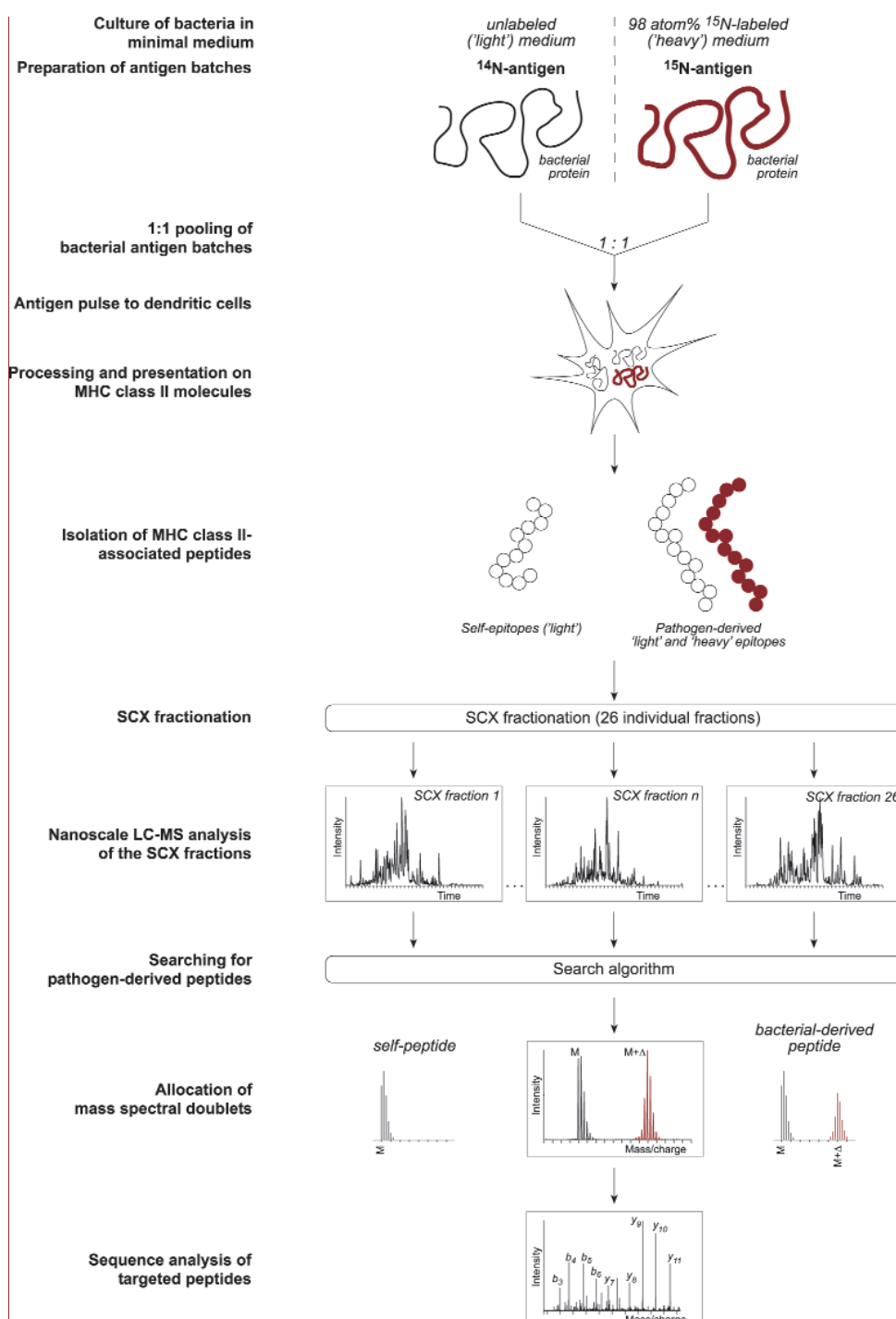


Figure 1. Experimental design for the allocation and identification of naturally processed and MHC class II-presented peptides that stem from the pathogen. By pooling batches of ¹⁴N-labeled ('light') and uniformly ¹⁵N-labeled ('heavy') antigens just prior to pulsing dendritic cells, all pathogen-derived epitopes will appear as mass spectral doublets in the LC-MS analysis. The absolute mass difference in a doublet (i.e. 1.2% of the *m/z* value) is proportional to the number of N-atoms contained within the epitope.

Method

Pathogens (*N. meningitidis* serogroup B or *B. pertussis*) are cultured either in normal (¹⁴N) medium or in 98 atom% ¹⁵N-labeled medium. ¹⁴N- and ¹⁵N-labeled neisserial outer membrane vesicles^[1] or pertussis whole cells are prepared and mixed in a 1:1 ratio just prior to pulsing dendritic cells, homozygous for HLA-DRB1*0101 or HLA-DRB1*1501. HLA class II-associated peptides are then isolated^[2] and fractionated by Strong Cation eXchange (SCX) chromatography. Each fraction is analyzed by nanoscale LC-MS (NanoSeparations, The Netherlands, e: info@nanoseparations.com) and mass spectral doublets are extracted using the MS-ExeleratorTM software algorithm (MsMetrix, The Netherlands, e: info@msmetrix.com). Candidate epitopes are then identified by targeted nanoscale LC-MS/MS analysis and confirmed with synthetic analogues.

Results and discussion

- Naturally processed length variants of epitopes from the major abundant Porin A (PorA) outer membrane protein of *N. meningitidis* occurred at widely diverging densities (15-17,000 copies/cell) and were derived from 8 distinct regions of the molecule. The neisserial epitope repertoires of both HLA alleles partially overlap.
- Processing and presentation of PorA is nonrandom and natural polymorphism inside and outside the epitope regions play a significant role in the efficiency of processing and presentation of PorA epitopes to CD4⁺ T cells^[3].
- 5 aberrant epitope sequences provide unique evidence that the MHC class II route also enables *intra*- and *inter*molecular peptide splicing. The splicing determinants are a PorA core sequence and an additional group derived from the same PorA protein or from a host protein (figure 2). A glycine moiety is always involved at the site of noncontiguity, suggesting a proteolytic specificity that directs the generation of the splicing products.
- At least 5 epitopes presented on HLA-DRB1*0101 and originating from *B. pertussis* were identified at low abundances (5-175 copies/cell). These epitopes are derived from 3 distinct proteins.

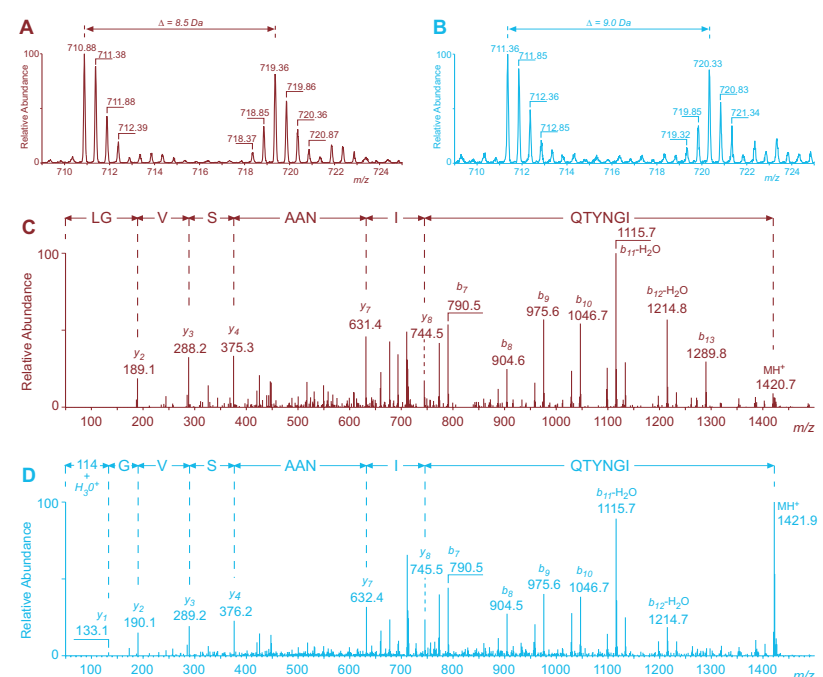


Figure 2. Doubly charged mass spectral doublets representing the native epitope IGNYTQINAASVGL (A) and the aberrant epitope IGNYTQINAASVG-[+114 Da] (B), containing 17 and 18 N-atoms, respectively. The MS/MS-spectra (C and D, respectively) show a striking similarity, except for the C-terminal amino acid residue that differs only by +1 Da. The unknown group has to contain 2 N-atoms that stem from the PorA molecule (as deduced from the mass difference between its ¹⁴N- and ¹⁵N-isomer), indicating an *intramolecular* peptide splicing product.

References

- Claassen *et al.*, Vaccine **14**, 1001-1008 (1996).
- Hunt *et al.*, Science **255**, 1261-1263 (1992).
- Meiring *et al.*, J. Immunol **174**(9), 5636-5643 (2005).