H.D. Meiring[1,¶] and E.C. Soethout[1,¶]
M.C.M. Poelen[1], J.A.M. van Gaans-van den Brink[1], J.A.M. Timmermans[1], A.J.R. Heck[2], A.P.J.M. de Jong[1], C.A.C.M. van Els[1]
[1] Netherlands Vaccine Institute, Bilthoven, The Netherlands
[2] Utrecht University, Utrecht, The Netherlands
¶ *Both authors made equal contributions to this work*
e: Hugo.Meiring@nvi-vaccin.nl

Identification of antigenic peptides utilizing dedicated metabolic leading strategies

Part I: MHC class I-associated peptides

Introduction

MHC molecules convey (intra)cellular fitness to immune effector cells by presenting both self- and nonself-peptides to T cells. Identification of the antigenic peptides is important to gain insight in the natural processing and presentation of these peptides and to understand the immune mechanisms in infectious diseases. The analytical challenge in the identification of only the infection-related epitopes lies in the discrimination between those peptides and the peptides not necessarily associated to the particular infection. To facilitate the unambiguous recognition of disease-related MHC class I-associated peptides, we developed a strategy of Stable Isotope Tagging of Epitopes (SITE, figure 1).

Method

Antigen presenting cells (B cells or dendritic cells, homozygous for HLA-A*0201 and/or HLA-B7) are grown in normal culture medium. One half of the batch is then virus-infected, either with measles virus (MV) or with respiratory syncytial virus (RSV), in a culture medium fortified with 50% of the labeled (*i.e.* 'heavy') and 50% of the unlabeled (*i.e.* 'light') amino acids, known as the HLA-specific anchor residues (figure 1). Just prior to peptide isolation, equal number of cells from both batches were pooled, thus affecting the binomial distribution of the isotope patterns for the stably expressed self-peptides only. HLA class I-associated peptides are then isolated by immunoaffinity purification, acid elution[1] and subsequent Strong Cation eXchange (SCX) fractionation[2]. Each fraction is analyzed by *nano*scale LC-MS analysis (NanoSeparations, The Netherlands, *e: info@nanosparations.com*) and binomially distributed isotope patterns are extracted using an in-house developed software algorithm. Candidate epitopes are then identified by targeted *nano*scale LC-MS/MS analysis.

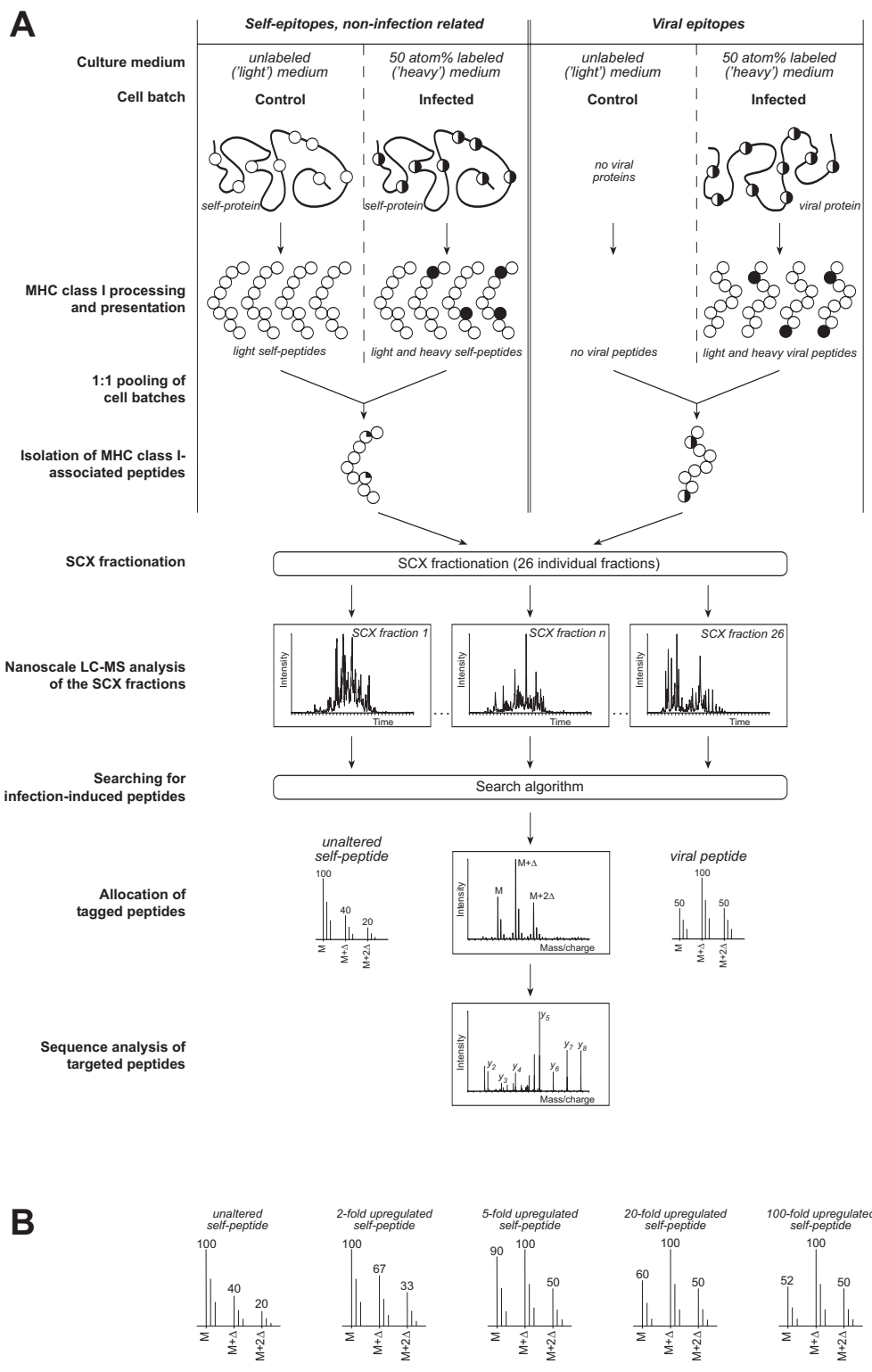

Figure 1. (**A**) Experimental design for the allocation and identification of virally induced MHC class I-associated peptides using the SITE strategy. The binomial distribution of the isotope patterns is illustrated for an antigenic peptide containing up to two labeled residues (visualized by filled circles). (**B**) Theoretical isomer distributions of self-peptides containing up to two 'heavy' amino acids, from pooled uninfected and virus-infected cells grown in 100% 'light' and in 50% 'light', 50% 'heavy' medium, respectively.

Results and discussion

- A shared component in the host antiviral response was found after MV or RSV infection by the increased presentation of the epitope TLIDLPGITRV (*source protein*: γ-interferon-induced protein MxA, *function*: inhibition of RNA virus replication).
- SITE allowed the identification of a new viral T cell epitope GLASFILTI after MV infection (*source protein*: nucleoprotein, *function*: replication of the viral genome).
- SITE allowed the identification of the RSV-derived epitopes KLIHLTNAL and KARSTPVTL (figure 2), the first naturally processed and presented epitopes to be described for RSV[3]. These epitopes stem from the RSV-NS and -F proteins, respectively, to which $CD8^+$ T cell responses were reported in children who had just recovered from severe RSV infection[4].
- 11 out of 14 upregulated self-epitopes presented on HLA-B7 after RSV infection stem from signal peptides of the HLA-A, -B or -C α-chain[3].
- The SITE strategy enables the absolute quantification of *de novo* synthesized or upregulated peptides (figure 1B).

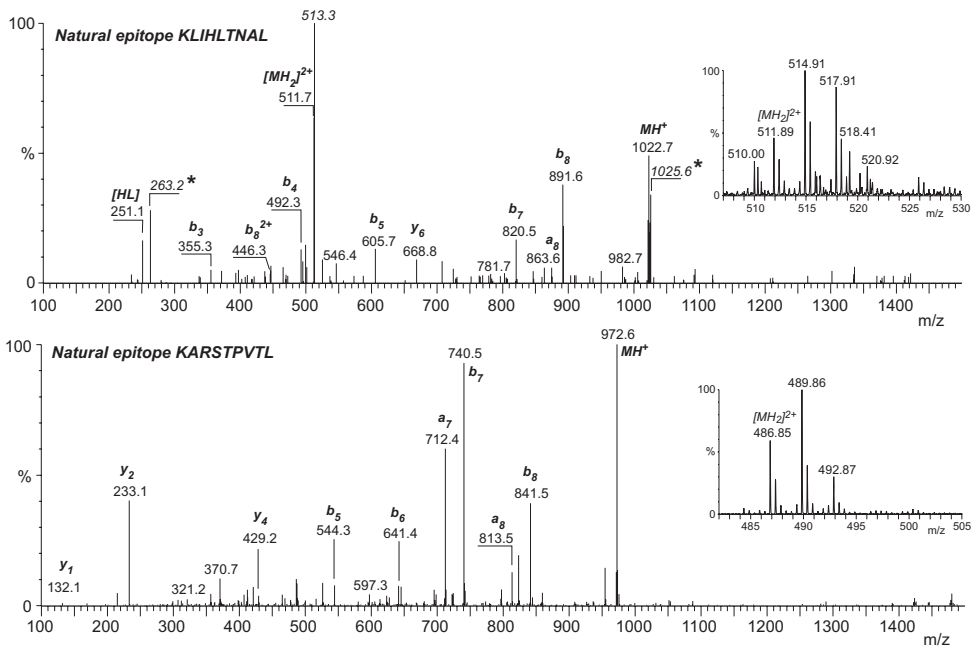

Figure 2. MS/MS spectra of the RSV-originating epitopes KLIHLTNAL and KARSTPVTL, including their respective binomially distributed mass spectral isotope patterns (insets).

References

1. Hunt *et al*., Science **255**, 1261-1263 (1992).
2. Meiring *et al*., Proceedings 51st ASMS conference, Montreal, Canada, abstract# 293 (2003).
3. Meiring and Soethout *et al*., Mol Cell Proteomics **5**, 902-913 (2006).
4. Brandenburg *et al*., J Virol **74**, 10240-10244 (2000).