Pre-mRNAs is the Origin of Neoantigens for the major histocompatibility complex class I pathway

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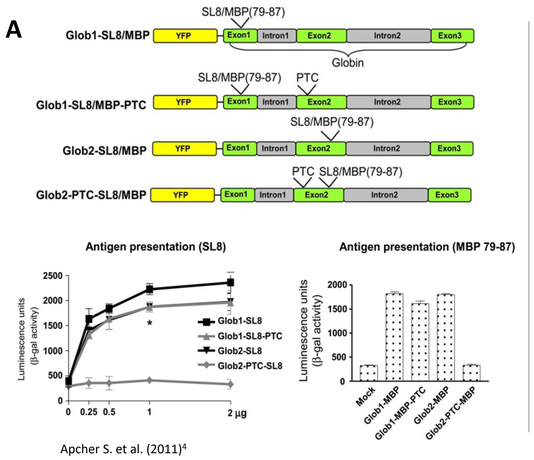
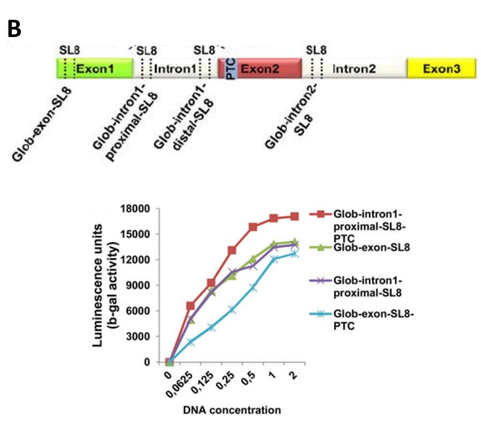
BACKGROUND

The capacity of the immune system to detect peptides presented on major histocompatibility (MHC) class I molecules forms the basis for CD8 T cells to distinguishing self from non-self antigens. CD8 T cells are "educated" in the thymus to be tolerant towards endogenous antigens and it has been puzzling how the immune system generates tolerance towards alternative tissue-specific splicing products. One can imagine that the thymic cells express and translate all possible splice variants *or* that source of antigenic peptides substrates (APS) is derived from pre-spliced transcripts. In the latter scenario, the immune system would not care about whatever splicing taking place in whatever tissue. Some years ago we made the observation that the latent Epstein-Barr viruses targets mRNA translation to evade the immune system and that mRNAs transfected into cells produce APS up to two ours following transfection whereas full length proteins are produced as long as the mRNA is present. We could later show that mRNAs targeted for the NMD pathway produce as much APS as the properly matured mRNAs. Even more surprising was the observation that APS are also efficiently produced from introns. It should be noted that CD8 T cell assay are extremely sensitive and only a few peptides on the MHC class I molecules is required for detection and we needed extensive mass spectrometry analysis to the detect APS. In order to ensure that these observations were not due to in cellulo artefacts we generated the Hbb mice that carries the SL8 class I epitope in the second intron of the Beta-Globin gene. More recent works show that the Hbb animals are tolerant against the SL8 and, thus, pre-spliced messages is indeed the source for class I peptides. This has some interesting implications. Firstly, it suggests that the first peptide substrates derived from any transcripts are selected for the MHC class I pathway which ensures rapid detection of virus-infected cells. Perhaps more intriguing are the implications for mRNA translation. There is, thus, no doubt that introns are translated and our collective data (not all shown here) show that the canonical translation of full length proteins and APS is distinguishable, supporting the notion that more than one mRNA translation even takes place. But what is really interesting and makes, at least some, members of the mRNA translation community have a proper fit is the suggestion that this translation event takes place in the nucleus. If you are curious, and brave, enough to consider nuclear translation I am happy to discuss and show what other data we have so you can make up your own mind!

In vitro studies on Pioneer Translation Products (PTPs) for the MHC class I pathway

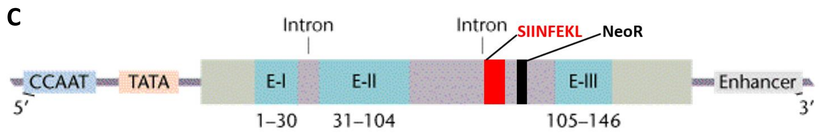


Apcher S. et al. (2011)[4]



Apcher S. et al. (2013)[5]

ANIMAL MODEL



Hbb mice:

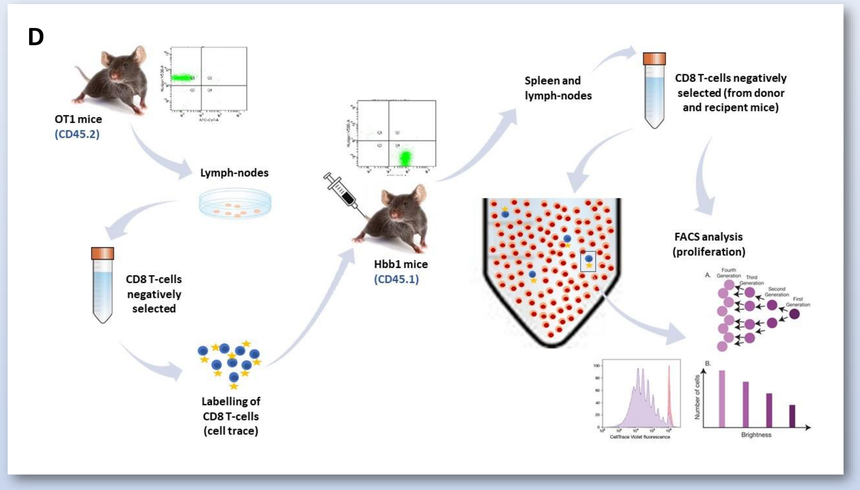
- SIINFEKL in intron 2 of β-globin gene Introduced by homologous recombination
- Two strains:
  - Hbb1 (T cells expressing CD45.1)
  - Hbb2 (T cells expressing CD45.2)

FIGURE LEGEND:

Data from *in vitro* studies on PTPs show that a major source of class I antigenic peptides is produced during the pioneer round of mRNA translation (Figs. A, B). Figure A (left) shows that antigens (SL8 or MBP) can be presented to CD8+ T cells even when the β-globin message is target for nonsense-mediated decay by a premature termination codon (PTC). Figure B (right) shows that antigenic peptides origin from pre-spliced RNAs. SL8 peptide sequence introduced to intron 1 or 2 of β-globin gene is a source of antigenic peptides presented to CD8+ T cells.

An animal model in which the SL8 sequence was inserted in intron2 of β-globin gene (C). An antigen presentation assay was performed *in vivo* (D). CD8+ T cells specific to the SL8 epitope were taken from the transgenic OT-1 mice, labelled and injected to Hbb mice. CD8+ T cells from Hbb mice were collected after 3 days and analysed by FACS towards the level of dye - cell trace violet. If the T cells detect the antigens they will proliferate and consequently loose dye. In this experiment a 2-fold increase in proliferation of CD8+ T cells from Hbb mice was noticed compared with wild type animals .

IN VIVO ANTIGEN PRESENTATION ASSAY



RESULTS

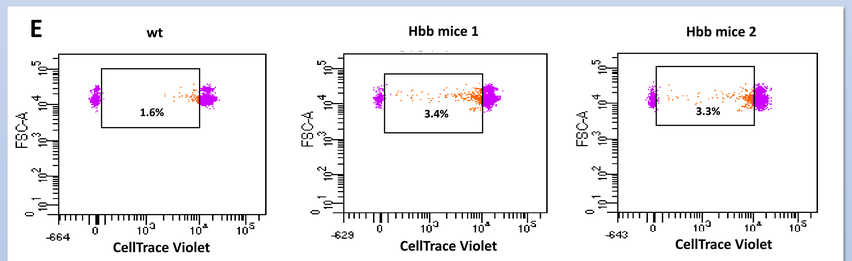


Figure E. OT-1 derived CD8+ T cells proliferate *in vivo* after injection to C57BL/6-Ly5.1 hbb CD45.1 mice. FACS analysis of stained OT-1 derived CD8+ T cells isolated from Hbb mice. Cells from 2 homozygous mice (hbb mice 1 and 2) showed higher level of proliferation (3.4% and 3.3% of total OT-1 derived CD8+ T cells, respectively) compared to a wt animal from which 1.6% of OT-1 derived CD8+ T cells proliferated.

CONCLUSION & PERSPECTIVES

Pre-spliced mRNAs translated in the nuclear compartment is the source of antigenic peptide substrates for the MHC class I pathway.

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