The role of alternative sources of antigenic peptides for the major histocompatibility complex class I in the formation of immune responses and immune tolerance

ABSTRACT

The major histocompatibility (MHC) class I pathway plays a critical role in distinction between healthy cells and those that are malignant or infected by viruses or other pathogens. A key component of the immunosurveillance is the scanning by CD8+ T cells for the presentation of non-self peptides on MHC I molecules. For a long time it was a,ssumed that MHC I immunopeptidome represents peptides derived from proteolytic degradation of so-called retired full-length proteins by the 26S proteasome. However, decades of studies indicated that proteasomal degradation of full length proteins is not the source of MHC I antigenic peptides and other sources have been proposed. The search into antigenic peptide origin has shifted from degradation of full length proteins towards synthesis, of alternative peptides. But how alternative peptide substrates are produced and their physiological role in immune surveillance is still poorly understood. In this study we show that an MHC class I epitope (SL8) inserted in the second intron of the β-globin gene in a C57BL/6 mouse (HBB) generates immune tolerance. Introduction of SL8-specific CD8+ T cells derived from OT-1 transgenic mice in HBB animals resulted in a 3-fold increase in OT-1 T cell proliferation, as compared to wild type animals. The growth of MCA205 sarcoma cells expressing the intron-derived SL8 epitope was suppressed in wild type animals compared to HBB mice. Immunisation with SL8-pulsed and LPS-activated DCs revealed reduced numbers of endogenous SL8-specific CD8+T cells in HBB mice as compared to WT controls. The pre-spliced β-globin message was detected in the light polysomal fraction and introducing stop codons identified a non-AUG initiation site between +228 to +255 nts upstream of the SL8. Isolation of ribosome footprints confirmed translation initiation within this 27 nt sequence. Furthermore, treatment with splicing inhibitor shifts the translation of pre-spliced mRNA to monosomal fractions and resulted in an increase of intron derived peptide substrate as shown by polysome profiling and cell imaging. These

results show that non-AUG initiated translation of pre-mRNAs generates peptides for MHC class I immune tolerance and help to explain why the products of alternative tissue specific splicing are tolerated by the immune system.