Title: Origin of antigenic peptides in MHC class I pathway

Abstract

A key component of the immune systems' capacity to distinguish between self and non-self is the presentation of peptides on major histocompatibility complex class I (MHC-I) molecules. Non-self-peptides derived from pathogens or mutated self-proteins are recognized by cytotoxic $CD8^+$ T cells leading to the destruction of the presenting cell. The presentation of intron-derived peptides on MHC-I molecules has raised the question of the origin of antigenic peptides for the MHC-I pathway. A better understanding of the origin of neo-antigens will lead to a better comprehension of viral and cancer immune evasion. For my Ph.D. study, I have focused on two different sources of antigenic peptides. Firstly, autophagy as a protein degradative mechanism, and secondly, pioneer translation products derived from pre-spliced mRNA.

First mechanism

Autophagy has an essential role in cellular homeostasis and can help rid the cells of harmful protein aggregates accumulation that can cause several diseases such as neurodegenerative disorders. Immune response towards cells carrying protein aggregates is relatively unknown and there is limited evidence for autophagy processing of antigenic peptides for the MHC class I pathway. To assess MHC-I antigen presentation of autophagy-derived antigenic peptides, we used $CD8^+$ T cells (OT-1) that specifically recognize the chicken ovalbumin (OVA) SL8 epitope (SIINFEKL) presented on the murine Kb MHC-I molecules. We evaluated potential substrates for autophagy processing by the ovalbumin-SIINFEKL sequence fusion to the aggregate-prone polyglutamine (PolyQ) and the Epstein Barr Virus-encoded EBNA1 sequences. Suppressing autophagy by knocking down Atg5 and Atg12 did not affect the presentation of peptides derived from the EBNA1 protein, whereas it reduced the presentation of antigenic peptides derived from OVA, or OVA fused to the aggregate-prone PolyQ sequence. Surprisingly, fusing ovalbumin to the immune-evasive glycine-alanine repeat (GAr) of EBNA1 (GAr-OVA) prevented the presentation of peptides from OVA. These data suggest a substrate-dependent presentation of antigenic peptides for the MHC class I pathway via autophagy and illustrate a novel virus-mediated mechanism for immune evasion of autophagy-dependent antigen presentation.

Second mechanism

Over time, the assumption that antigenic peptides are solely derived from the degradation of “old” full-length proteins has been replaced with the postulation that antigenic peptides are also derived from newly synthesized peptides by a specific non-canonical alternative translation event that occurs before mRNA splicing. This would explain the presence of intron-derived peptides on MHC-I molecules. In support of this, we observed that expressing the SIINFEKL sequence in the second intron of the β-globin gene triggered OT-1 $CD8^+$ T cell proliferation. Using the proximity ligation assay (PLA), we observed an increase in SIINFEKL peptides from pre-spliced mRNAs following treatment with the Isoginkgetin splicing inhibitor. To start the characterization of this alternative translation complex, we used polysome fractionation to identify ribosomes on pre-spliced mRNAs. The pre-spliced β-Globin mRNA is found on light polysomes, as opposed to the spliced β-Globin mRNA that is present in the heavy polysomal fractions. The data further supports the notion that antigenic peptides are derived from the translation of pre-spliced mRNAs.