Gene optimization can generate cryptic epitopes that induce T cell responses

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Codon optimization of nucleotide sequences is a widely used method to yield high levels of gene expression for gene therapy approaches or to increase the immunogenicity of nucleic acid-based vaccines. To overcome poor expression levels of human papillomavirus (HPV) E6 and E7, which play a causative role in cervical cancer, we codon-optimized both genes. E6 and E7 mRNA-pulsed dendritic cells were used to stimulate and screen for antigen-specific T cells. Candidate T cell receptor (TCR) genes were identified and cloned into a retroviral vector to characterize them with TCR-transduced peripheral blood lymphocytes (PBLs). We successfully isolated four TCRs with different V-alpha and V-beta chains from two unrelated donors, which recognized target cells harboring the codon-optimized E7 gene but not the wild type sequence. Epitope mapping surprisingly revealed a cryptic epitope at the 5' end of the codon-optimized E7 as target antigen for all TCRs. The -1nt alternative reading frame from the codon-optimized sequence translated for an artificial protein sequence, which is not encoded by the wild type E7 sequence due to the exchange of wobble nucleotides in the +0 reading frame for optimal codon usage. The resulting cryptic epitope is unrelated to any human or viral sequence, performs at excellent levels in epitope prediction servers regarding proteasome cleavage, TAP transport and binding affinity to the TCR restriction element HLA-B*27:05 and thus is an highly immunogenic antigen.

Mechanisms behind the phenomenon of protein translation initiated else but by the classical ATG start codon might be translational initiation noise or the active generation of defective ribosomal products (DriPs).

In conclusion, codon optimization of a nucleotide sequence can generate immunogenic cryptic epitopes from alternative open reading frames, which induce T cell responses that may lead to the rejection of engrafted cells after gene therapy or false positive immune responses after DNA vaccination. This novel finding is of great importance for the field of gene therapy and vaccination when designing nucleotide-based constructs to avoid unwanted immunogenic side effects.