Title: Molecular Analysis of NFAT/ICER Repressor Complexes and their Role in nTreg-cell mediated Suppression

To obtain insight into the interaction of inducible cAMP early repressor (ICER) and nuclear factor of activated T cells (NFAT) we will determine the structure of the complex formed between the basic leucine zipper (bZIP) domain of ICER and rel similarity domain (RSD) of NFAT as well as the ternary complex of the two protein fragments in the presence of DNA for further functional studies. The most pronounced ability of ICER to associate with NFATc2 was observed at the sites with the highest DNA binding efficiencies to both proteins, particularly at the site in position (–160) of the interleukin–2 (IL-2) promoter also known as the CD28 responsive element (CD28RE), and to a lesser extent to the NFAT site in position of (–45). Interestingly, the NFAT site in position (-45) tends to show a stronger binding of ICER to NFATc2 in the NFATc2-RSD/ICER complex compared to ICER itself. Because the NFAT (-45) site of the IL-2 promoter is the only one of the five sites examined without an adjacent AP-1 site, the finding that this site can form an NFATc2-RSD/ICER complex suggests that ICER itself may tether to NFAT by protein-protein interaction in addition to protein-DNA interactions. This notion is further supported by immunoprecipitations (IPs) and GST-pull downs where ICER interacts with short isoform of NFATc1 in the absence of DNA.