

Abstract

We tried exploring the probability of GPR114 serving as a memory CD8 T cell marker. For that we purified the protein in soluble and refolded form. Then the antibodies against this protein were generated in mouse. The specificity and sensitivity of these antibodies was checked by ELISA and western blotting. To prove it as a marker, its expression was checked on different immune cells like naïve CD4 and CD8 T cells, stimulated CD8 T cells using these anti-gpr114 antibodies. We found that naïve CD8 T cells express this protein and not stimulated CD8 T as well as not naïve CD4 T cells, as the expression was checked by western blot. Our next step is to check its expression on memory CD8 T cells using various immunological and molecular biology approaches followed by in vivo studies. We attempted at studying the effects of Russell Viper venom on immune cells. For that we isolated the different proteins present in venom using gel filtration chromatography and collected five different peaks of proteins. We then tried to check the effect of each protein as well as the whole venom on immune cells. We found that fraction one from gel filtration of intact venom is most effective in causing apoptosis as compared to other fractions. Some of the fractions are found to induce necrosis in immune cells. Intact venom was found to be less efficient in inducing apoptosis in immune cells when compared with fraction one probably because of the presence of proteins that have pro and anti-apoptotic properties. This implies (our estimation is) that the whole venom contains some proteins or molecules that are shielding the cells from apoptosis/necrosis causing proteins. Future studies will focus on characterizing and identifying the venom components using Mass Spectrometry. The overall aim is to devise strategies to counteract their effects using immunotherapeutics. Additional work focused on cloning and characterization of some of the biologicals from bacterial system but we were not able to clone the polymerase of *P. furiosus* and lipase of *T. maritima*, might be due to various problem. For characterization of Cel CCA, the purified protein was found to have helical and betasheet structure and is in monomeric form of size 3nm as observed by DLS analyses. The protein has inverse relation with the concentration of GuCl and fluorescence intensity of protein Cel CCA.