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Grant Title: Identification and characterization of the carbohydrate-specific T-cell receptors

Abstract:

We previously identified CD4+ T-cell clones specifically recognize the carbohydrate portion of glyco-conjugate vaccine (III-OVA and III-TT) in the context of major histocompatibility complex (MHC) class-II molecules expressed on antigen-presenting cells (APCs). Therefore, isolation and characterization of the carbohydrate antigen-specific T-cell receptors (TCRs) are important for understanding the mechanisms underlying the action of glycol-conjugate vaccines. An improved 5'-RACE protocol was used for identifying TCR genes from one carbohydrate antigen-specific T-cell (Tcarb) clone (2A2). OVA-specific TCR genes were also cloned from splenocytes of DO11.10 transgenic (Tg) mouse by using a similar approach but with different set of primers. These identified TCR genes were cloned and transduced into 58-/- (TCRαβ-double negative) cell line, a derivative of BW5147 thymoma, by using retrovirus. Two TCR-α and two TCR-β genes were identified from 2A2 cells. After pairing of different TCR-α and β genes, four 58-/- cell lines transduced with Tcarb-derived TCRs were generated for functional assay. OVA-specific TCR-transduced cell line, as positive control, was generated and analyzed in parallel. Although expression of respective TCR was successfully confirmed by flow cytometry, there was no detectable IL-2 production in any TCR-transduced 58-/- cell lines after stimulation with respective antigen presented by professional APCs, indicating that some improvements are required to detect function of the Tcarb-specific TCR in the future.