

## SARAT DALAI

### Immunopathology

#### **B Cells Induce Anergy in Memory CD4<sup>+</sup> T Cells**

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Induction of tolerance in memory T cells is an important phenomenon in preventing responses against peripheral antigens in autoimmune diseases. Memory T cells are less susceptible to anergy whereas naive T cells can be anergized readily. We have previously reported that presentation of low densities of agonist peptide/MHC II (1-10 complexes per antigen presenting cells, APCs) induced anergy in T cells *in vitro* (Korb, L. C. *et al. J. Immunol.* 162:6401,1999) and in memory T cells *in vivo* (Mirshahidi, S. *et al., J. Exp. Med.* 194:719, 2001). More recently, we have demonstrated that TCR engagement is the predictor of T cell activation or anergy. In other words, presentation of low density of peptide/MHC by antigen presenting cells lead to engagement of a lower number of TCR and thus activation of TCR initiated signaling cascade leading to T cell inactivation (Mirshahidi, *et al, J. Immunol, in press*).

Induction of anergy in memory T cells has profound applications in treatment of autoimmune diseases and in control of transplant rejection. It is critical to identify APCs that can present T cells in a tolerogenic fashion. Although B cells anergized T cell clones in our simplified *in vitro* system, determination of the responsible antigen presenting cells to interact with memory T cells and rendered them anergic *in vivo* was challenging because of the complexity of the system. Dendritic cells are potent and primary APCs for initiation of an immune response. They also have been shown to be involved in tolerance induction *in vivo*, although through unknown mechanisms. When low doses of peptide antigen is offered *in vivo*, it is feasible to assume that dendritic cells are the likely APC candidates because of their high levels of MHC II expression. Thus, we designed an experimental system to test the nature of APC involved in tolerogenic presentation of peptide to T cells. The following experiment using T cell transgenic system (D011.10) specific for ovalbumin peptide OVA<sub>323-339</sub> in complex with I-A<sup>d</sup> was designed: D011.10 T cells were transferred to BALB/C recipients followed by immunization with cOVA<sub>323-339</sub> peptide in adjuvant. Five weeks later, to allow for generation of memory T cells, groups of mice received low doses of peptide in adjuvant as tolerogen. After 9 days, mice were sacrificed and examined for T cell responses. We observed that transgenic CD4<sup>+</sup> T cell proliferation was arrested in groups of mice that had received OVA<sub>323-339</sub> at 0.05-0.005 nmoles. This was tested by *in-vitro* CFSE dilution assay and by <sup>3</sup>H-Thymidine incorporation. For **in-vitro** tolerization of T cells, groups of mice were immunized subcutaneously with different doses of peptide in IFA and two days later, which is required for tolerogenic presentation of antigen (based on T cell proliferation and antigen specific cytoplasmic staining of IL-2 and IFN- $\gamma$ ), APCs were isolated from the inguinal lymphnodes and were cultured with memory T cells obtained from the mice that had received single dose of immunization. This was done in order to tolerize memory T cells *in vitro* by *in vivo* pulsed APCs. We observed *in vivo* pulsed total APCs (LN cells devoid of T cells) could tolerize memory T cells *in vitro*. Preliminary results showed that B cells might be the likely APCs to tolerize memory T cells. We are pursuing our efforts to find whether dendritic cells under similar condition could do the same. The uniqueness of this study compared to our earlier studies is that we look for the response at the individual cell level as opposed to global response. Also we are working on tolerizing memory T cells *in vivo* by injecting antigen pulsed APCs *in vitro* instead of antigen directly.