

**Applicant's Name:** Kedar Narayan, B.A.

**Applicant's Division:** Immunology

**“Direct Visualization of Staphylococcal Enterotoxin A Induced Clustering of HLA-DR1 in B cells”**

Kedar Narayan, Edward Perkins and Scheherazade Sadegh-Nasseri

**Background:** Staphylococcal Enterotoxin A (SEA) is a bacterial toxin and a powerful T cell mitogen that causes non-specific immune hyperactivation in the host. Biochemical and structural studies have shown that SEA possesses two binding sites for two corresponding sites on the MHC class II molecule HLA-DR1 (DR1). However, the stoichiometry of the SEA binding to DR1 remains unclear, and direct binding of SEA to DR1 has not been visualized. We addressed the possibility that SEA binding to adjacent DR1 molecules on the B cell surface may crosslink DR1, allowing for formation of daisy-chain like DR1-SEA multimers, and immune activation.

**Results:** Here, we show that SEA can form aggregates with DR1 in solution in a manner that indicates utilization of both binding sites on the molecules. Further, using Forster Resonance Energy Transfer (FRET) studies, we show that SEA but not SEH (which has only one DR binding site) can cause clustering of surface HLA-DR1 on freshly isolated purified B cells from DR1 transgenic mice in a  $Zn^{2+}$  and dose dependent manner. In addition, we have used Electron Microscopy to gauge the extent of surface DR1 clustering on B cells. We show that there while is basal level random clustering of DR1 on the surface of B cells even in the absence of enterotoxin, addition of SEA but not SEH increases the degree of order of this clustering.

**Conclusions:** These results provide visual evidence that strongly support the possibility that SEA may crosslink DR1 molecules to form ordered DR1 clusters on the B cell surface. These clusters are formed independent of T cells, and may describe a new facet of the mechanism by which SEA induces immune activation.