Determining the Specificity of a Multivalent Polymeric Antigen-Specific Immunotherapy for Multiple Sclerosis

Brittany L. Hartwell, Joshua O. Sestak, Bradley P. Sullivan, Jun Chen, David Moore, Heather Shinogle, Teruna Siahaan, Cory Berkland
University of Kansas, Lawrence, KS

Introduction: Multiple sclerosis (MS) is an autoimmune disease that manifests when myelin sheath undergoes autoimmune attack. Many current therapies for MS act systemically on the general immune response, yielding limited therapeutic efficacy and adverse side effects [1]. Soluble Antigen Arrays (SAgAs) are an antigen-specific immunotherapy designed to be safer and more effective by targeting immune cells that trigger the autoimmune response – specifically, by interrupting the immunological synapse between professional antigen presenting cells (pAPC) and T cells [2]. The SAgA design consists of a hyaluronic acid (HA) polymer backbone complexed with two peptides presented in a multivalent array. The two peptides are disease-specific autoantigen (proteolipid protein peptide epitope, $PLP_{139-151}$) and ICAM-1 inhibitor peptide (LABL), intended to target the B cell receptor and ICAM-1, respectively. Codelivery of antigenic peptide and cell adhesion inhibitor peptide on HA is necessary for SAgA treatment of EAE, the murine model of MS [3]. *In vitro* kinetic studies were performed with pAPC and fluorescently-labeled SAgA and its design variants to elucidate the therapeutic mode of action on a cellular level. It was hypothesized that SAgA would exhibit specific binding with B cells (a type of pAPC) dependent on the identity and density of conjugated peptide, largely driven by the antigenic peptide (PLP).

Methods: HA (16 kDa) was first labeled with fluorescein isothiocyanate (FITC) in order to quantify and visualize binding (labeled polymer denoted as *HA). *HA was then conjugated with aminooxy-LABL and -PLP, with a target valency of approximately 10 peptides per HA backbone, to form a multivalent array. Homopolymers (*HA_{PLP} and *HA_{LABL}) were grafted with one of these peptides, and a heteropolymer (*$SAgA_{PLP:LABL}$) was co-grafted with both peptides. Raji B cells were cultured, then activated with TNF-α and primed with PLP peptide 24 hours prior to experiments.

Binding and competitive dissociation studies were performed on a flow cytometer (Beckman Coulter MoFlo XDP Cell Sorter). To observe maximum equilibrium binding, cells were mixed with labeled polymer arrays (dosed by constant peptide molarity) immediately before injection on the flow cytometer. The sample was run until maximum equilibrium was reached (B_{max}), at which point 40-fold excess unlabeled reagent was added to competitively dissociate the bound labeled array. Data acquisition was stopped after dissociation steady state (representing non-specific binding) was established. Homopolymers were dissociated by competition with corresponding individual components (*HA with HA, *HA_{LABL} with LABL, and *HA_{PLP} with PLP) to determine specificity of binding. Heteropolymer *$SAgA_{PLP:LABL}$ was dissociated by competition with various unlabeled reagants (HA, PLP, or LABL) to determine specific binding. Nonlinear regression and statistical analysis were performed using ANOVA followed by Tukey's post-hoc test with $\alpha=0.05$ to determine statistical significance.

Live cell imaging of binding between cells and labeled polymer arrays was observed on an Olympus IX81 inverted epifluorescence microscope using the CellASIC ONIX Microfluidics Platform and M04S switching plate (EMD Millipore, Billerica, MA).

Results: Flow cytometry binding studies showed that *$SAgA_{PLP:LABL}$ exhibited the highest B_{max} in primed and activated cells compared to the homopolymers ($p<0.01$) and *HA ($p<0.0001$) (**Figure 1**). Competitive dissociation with homopolymers showed that *HA_{PLP} and *HA_{LABL} both exhibited significantly higher specific binding than *HA ($p<0.01$). Competitive dissociation with heteropolymer *$SAgA_{PLP:LABL}$ showed that PLP was responsible for the highest extent of specific binding, significantly higher than both HA and LABL ($p<0.01$). Fluorescence microscopy images showed localized binding of *HA, homopolymers, and *$SAgA_{PLP:LABL}$ on the cell surface.

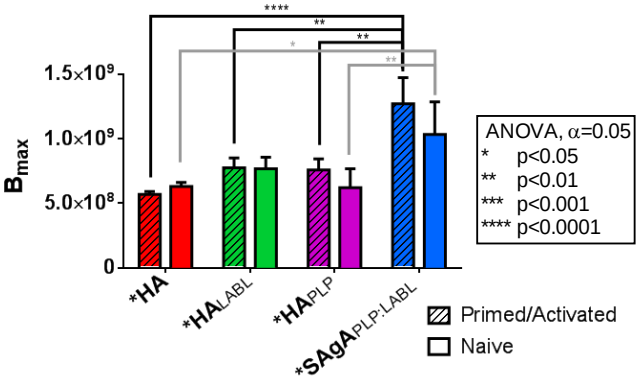

Figure 1. B_{max} of various polymer arrays with Raji B cells. Data reported as mean plus standard deviation (n=3)

Conclusions: SAgA molecules bind Raji B cells in a specific manner and conjugated peptides (LABL and PLP) increase binding. Specificity is primarily attributed to PLP. Ongoing studies are exploring the binding specificity of SAgAs with other cell types known to be involved in the immunological synapse, including mixed murine splenocyte populations.

References:

1. Wakerly B. Medicine Multiple Sclerosis. 2012;40:523-528.
2. Sestak JO. J Controlled Release. 2013;168:334-340.
3. Sestak JO. Mol Ther – Methods and Clin Dev. 2014;1:1-9.