VSoE Research Innovation Fund Report

Project Title: Targeted Delivery of Antigen for Novel Vaccines

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A. Summary of Experimental Results

Generation of target proteins (human and mouse DC-SIGN) We first generated recombinant target proteins (both human and mouse DC-SIGNs). The cDNAs encoding the extracellular domain (ECD) of DC-SIGN were PCR-amplified and cloned into pET302/NT-His vector. To facilitate the biotinylation reaction, we also introduced a biotin tag sequence into the N-terminal of DC-SIGN. The resulting plasmids were transformed to bacterial BL21 cells and IPTG was used to induce the expression of the recombinant biotin-DC-SIGN proteins for 4 hours, after which cells were pelleted by centrifugation and lysed in a denature buffer. Successful expression of proteins was confirmed by SDS-PAGE analysis (Fig. 1). The recombinant proteins were then purified through Ni-NTA column and refolded in a refolding buffer. To ensure that the biotin tag was appropriately biotinylated by endogenous BirA ligase of bacteria, we incubated the purified DC-SIGN proteins with streptavidin-coated beads. After extensive washing, proteins were eluted from beads by addition of biotin molecules. SDS-PAGE analysis showed that the purified proteins could bind to streptavidin, indicating that at least some fractions of recombinant proteins contained N-terminal biotin.

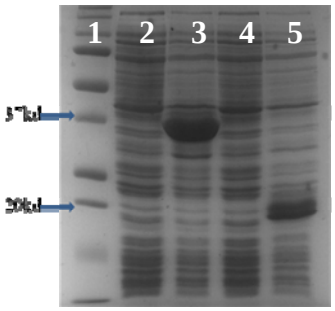

Fig. 1: SDS-PAGE analysis of various cell lysates. Lane 1: molecular weight marker; Lane 2: no induction (control); Lane 3: induced expression of human DC-SIGN; 4: no induction (control); 5: induced expression of mouse DC-SIGN

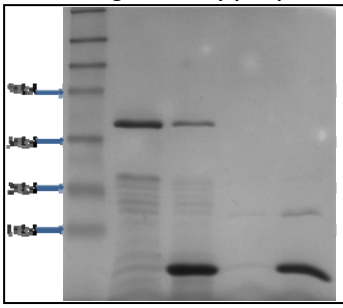

Fig. 2: SDS-PAGE analysis of purified DC-SIGN proteins. Lane 1: molecular weight marker; Lane 2: Ni-NTA-purified human DC-SIGN; Lane 3: Streptavidin bead-eluted human DC-SIGN; Lane 4: Ni-NTA-purified mouse DC-SIGN; Lane 5: Streptavidin bead-eluted mouse DC-SIGN;

Functional assessment of recombinant DC-SIGN proteins We next tested the binding function of recombinantly expressed DC-SIGN proteins. The DC-SIGN solution (200 nM) was coated onto a 96-well plate. HIV gp120-Fc fusion protein, a known DC-SIGN ligand, was added and incubated at room temperature for one hour. The plate was extensively washed and bound gp120 was detected by a HRP-conjugated anti-human Fc monoclonal antibody. We found that gp120-Fc could efficiently bind to the recombinant human DC-SIGN protein, suggesting that the carbohydrate binding domain (CBD) of DC-SIGN retains its binding activity. This binding function was further confirmed by a Biacore measurement.

mRNA display-based selection for protein ligands to DC-SIGN proteins For the selection for ligands, we exploited the 10Fn3 (tenth human fibronectin type III domain) scaffold, one of the popular scaffolds for protein design, to target DC-SIGN. The sequence of this scaffold is entirely of human origin, which is important for therapeutic use *in vivo*. It is a small and stable protein that can be expressed in a bacterial system at high levels. These intriguing features make 10Fn3 a good choice for selecting DC-SIGN binders for vaccines. Using the established and extensively characterized 10Fn3 library system in our laboratories, we amplified the 10Fn3

mRNA fusion library (size of 10^{13}) and used it to select against human DC-SIGN immobilized on avidin beads. After four rounds of selection, survived clones were co-selected to screen for binders with dual specificities (both human and mouse DC-SIGN) for several rounds. We

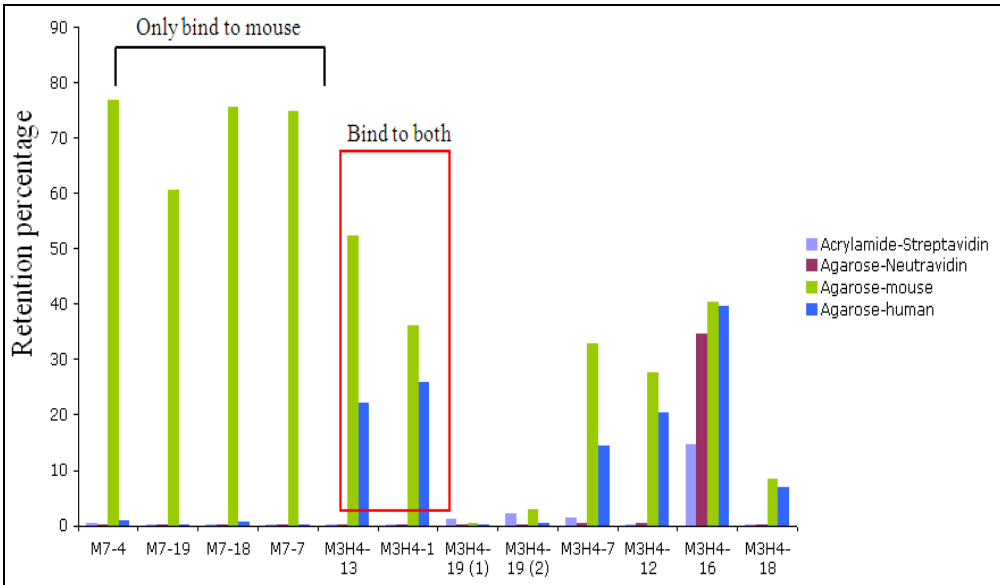

Fig. 3: In vitro selection to identify new binders to DC-SIGN. After 4-round selection against human DC-SIGN and 3-round co-selection against both human and mouse DC-SIGN, 12 independent clones were chosen for the radioactive equilibrium binding assay to confirm the specificity.

selected 12 independent clones to test their binding to DC-SIGN using a radioactive equilibrium binding assay (Fig. 3). There were four clones (M7-4, M7-19, M7-18, and M7-7) that could bind to mouse DC-SIGN and were two clones (M3H4-13 and M3H4-1) displaying the dual specificities. We then recombinantly expressed two selected binders (M7-4 and M3H4-13) and purify them with sufficient quantity for a flow cytometry binding assay, in which the binders were labeled with fluorescent dyes and then incubated with a cell line expressing human DC-SIGN (293T/hDC-SIGN). As shown in Fig. 4, both binders can specifically bind to DC-SIGN-expressing cells, while no binding can be seen for cells lacking the recognition receptor. Thus, using directed in vitro protein evolution by mRNA display, we were able to generate new binders that bound DC-SIGN receptor, a protein that is dominantly expressed in dendritic cells. We are in the process of testing the efficacy of these new binders to deliver immunogens for novel vaccine development.

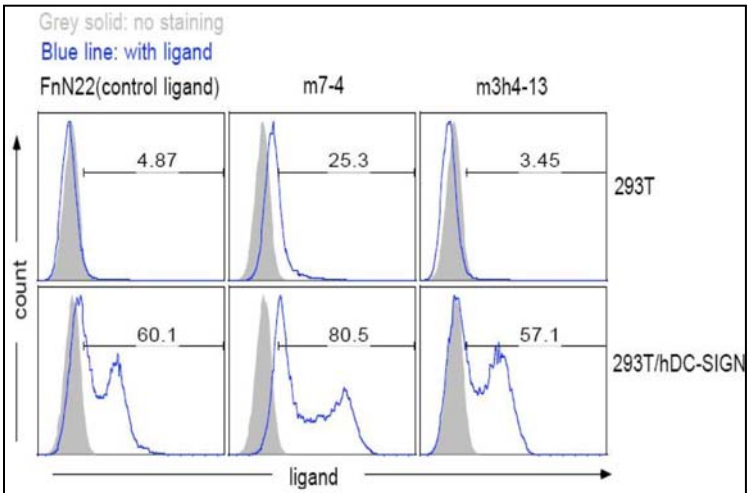

Fig. 4: A flow cytometry assay to confirm the binding of selected clones.

B. Fundraising

With the support of VSoE RIF fund, we were able to demonstrate the viable and powerful approach of using in vitro mRNA display to identify new molecules for vaccine delivery. These preliminary data and the collaboration established by this RIF fund allowed us to successfully obtain external funding from California Institute of Regenerative Medicine (CIRM) totaling

$951,104 for two years. We will continuously submit grant applications. We will monitor the proposal opportunity from the Bill and Malinda Gates Foundation for its global health program and will consider submitting proposals for vaccines against HIV/AIDS, Malaria, and Tuberculosis. We will also plan a proposal to the California University AIDS Research Program (UARP).