Characterizing a Novel Engineered Therapeutic Agent to Reverse Lupus Symptoms

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Abstract
Systemic Lupus Erythematosus (SLE) is a chronic inflammatory autoimmune disease that affects approximately 1 out of every 1000 individuals in the United States. Auto-antibodies are produced when improper destruction of apoptotic cells results in B-cell activation and differentiation. Since the auto-antibodies are secreted by mature B-cells, the complement system has long been a target of interest in treating SLE. Along with our collaborators, we have identified antibodies that have been shown to reverse the symptoms of SLE in a mouse model of lupus. In order to use this antibody as a potential therapeutic or imaging agent we have engineered a single chain variable fragment (scFv). After expressing the scFv in mammalian cells, subsequent purification resulted in high yields of pure protein. Binding affinities were measured and represent a valid approach to replace the much larger antibody.

Complement Introduction
-Part of the innate immune system that clears foreign particles and cells from the body
-Complement activation triggers a cascade in which end result is a potent inflammatory response [6]
-May also be responsible for eliminating lymphocytes that are reactive towards self, so complement compomise implicated in autoimmunity [3]

Systemic Lupus Erythematosus
-Autoimmune disease that attacks the body’s own tissues
-Abnormalities in apoptotic pathways directly correlated with the disease severity [2]
-Improper cell clearance after apoptosis and leftover debris picked up by antigen-presenting cells [5]
-No known cure for SLE, but immunosuppressants control over-active immune responses [7]

Results: Size Exclusion Chromatography

Results: Molecular Docking, Simulated Binding

Figure 5: Production of ScFv 3292 in 293T cells. His tagged ScFv protein engineered from mAb 3292 was purified by Ni-NTA affinity chromatography from supernatants of 293T cells transiently transfected with the expression constructs of these proteins. The purified ScFv 3292 was resolved by gel filtration chromatography on a Superdex 200 Increase column. The late elution volume is likely due to the compactness of the construct.

Figure 4: Binding affinity between GST-C3d and 3292. Kd value was calculated to be 0.42 ± 0.15 nM.

Figure 6: (left) Rigid-rigid dock of 3292 SWISS-MODEL (orange) with the known crystalized structure of Homo sapiens C3d (green). This dock had a HADDOCK score of 34.0 +/- 15.7. (right) Complex between C3d (green) bound to its natural complement receptor CR2 (red). The first residue of C3d has been colored purple for better ease of comparison.

Methods
Mammalian Cell Transfection:
Transfection media was prepared by placing DEMEM (no additives) in two tubes equal to 1/20 culture volume in each tube. To one tube 4ug of 3292 ScFv DNA per 5cm² of culture area was added. To the other tube PEI was added in a 1:4 DNA:PEI ratio. The tubes were equilibrated for 15 mins then the PEI tube was poured into the DNA tube. After another 15 minute incubation equal amounts of the DNA/PEI solution was added to each flask. Flasks were incubated overnight then media was removed, cells were washed with PBS, and 50 ml of Complete freestyle media (1% pen/strep) was added.

Purification of 3292 ScFv:
After incubation for 5 days in freestyle media, freestyle media was removed and clarified by centrifugation. supernatant was run through an equilibrated 5 ml His-trap nickel column. The column was washed with 5 column volumes of PBS with 20 mM imidazole. Protein was then eluted into 12 fractions of 1.5 ml using PBS containing 300 mM imidazole. Presence of protein was confirmed usingNanoDrop 2000C Spectrophotometer (Thermo Scientific).

FPLC purification:
Fractions which contained protein were concentrated down to 0.5 ml and injected into a 100 increase size exclusion column.

Expression and Purification of Human C3d:
C3d was expressed using the pEx system, thus tagging the C3d with Glutathione 5-Transferase (GST). Bacterial clones were incubated with shaking at 37 °C until OD600 was 0.3. Subsequently the culture was induced with IPTG and cultures were grown overnight at 25 °C. Bacterial cells were harvested by centrifugation, lysed by sonication, and supernatant passed over a GST affinity column followed by bulk elution using Glutathione. The concentrated eluent was further purified using FPLC as above.

BLITz Binding Analysis:
Protein samples were run through BLITzanalyzer (Fortelix) using the Simple Kinetics protocol. GST-C3d was bound to Anti-GST analyzer tips and then the same tip was subjected to binding analysis with the relevant protein. A serial dilution of the relevant protein was used in order to create binding graphs.

Future Directions
- More binding studies will be conducted in order to confirm the binding affinity between ScFv (3292 and 3298) and C3d
- Prepare crystal trays to begin process of crystallization and diffraction visualization

References

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