Characterizing a Novel Engineered Therapeutic Agent to Reverse Lupus Symptoms

Brianna Vigil and James M Kovacs
Department of Chemistry and Biochemistry
University of Colorado – Colorado Springs, CO, USA

Abstract

Systemic Lupus Erythematosus (SLE) is a chronic inflammatory autoimmune disease that effects 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 compromise implicated in autoimmunity [3]

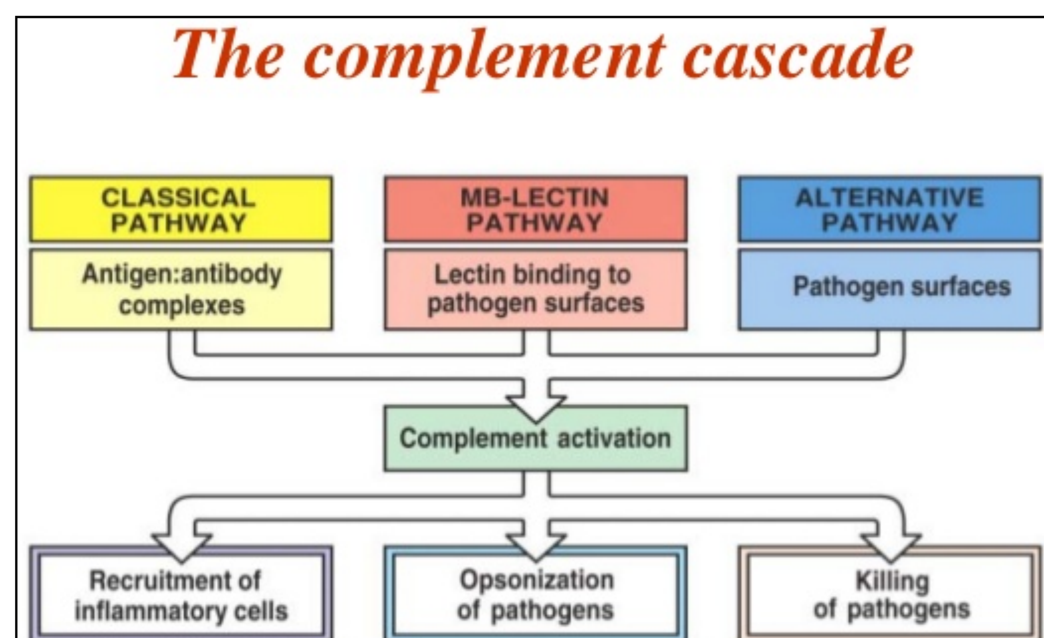

Figure 1: Complement Cascade (from Janeway et al., 2001)

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]

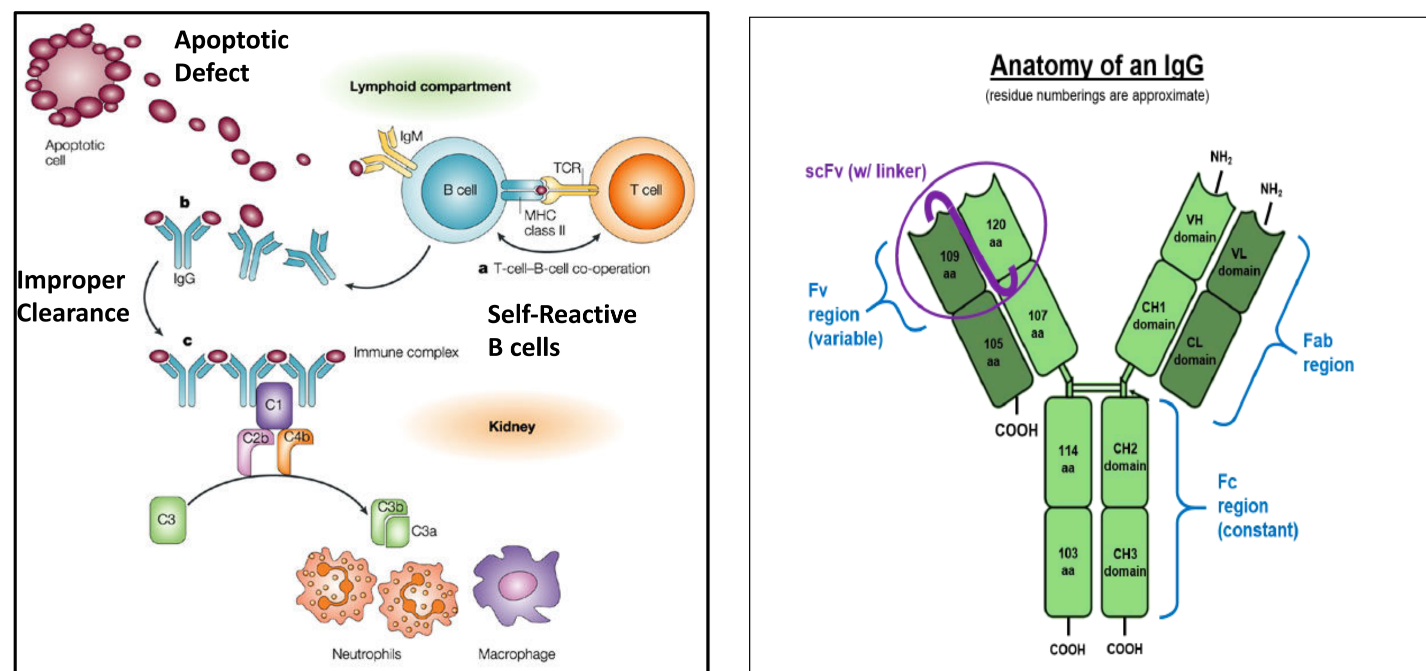

Figure 2: (left) Autoantibody-mediated pathogenesis of SLE (from Carrol, 2004). **(right)** Structure of IgG. Variable heavy and light chains are linked together. The Fab region and ScFv components can be seen. ScFv's are cheaper to produce and have better tissue penetration than traditional antibodies. (image adapted from Wikipedia)

Results: Size Exclusion Chromatography

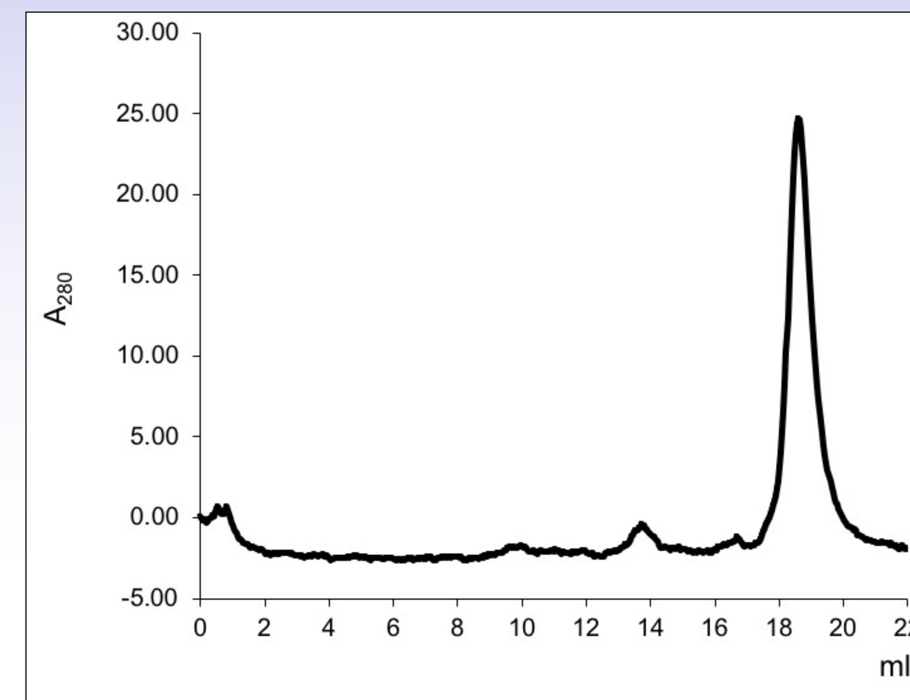

Figure 5. Production of ScFv 3D29 in 293T cells. His-tagged ScFv protein engineered from mAb 3D29 was purified by Ni-NTA affinity chromatography from supernatants of 293T cells transiently transfected with the expression constructs of these proteins. The purified ScFv 3D29 was resolved by gel filtration chromatography on a Superdex 200 Increase column. The late elution volume is likely due to the compactness of the construct.

Results: BLItz Binding Analysis

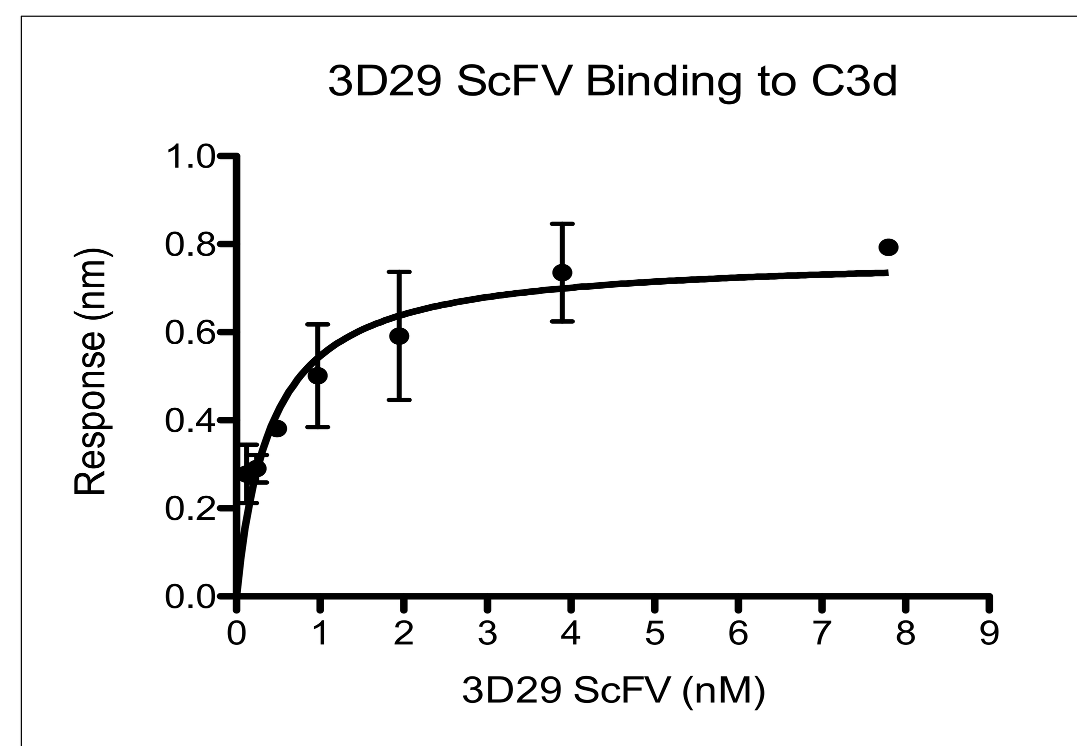

Figure 4: Binding affinity between GST-C3d and 3D29. K_D value was calculated to be 0.42 ± 0.15 nM.

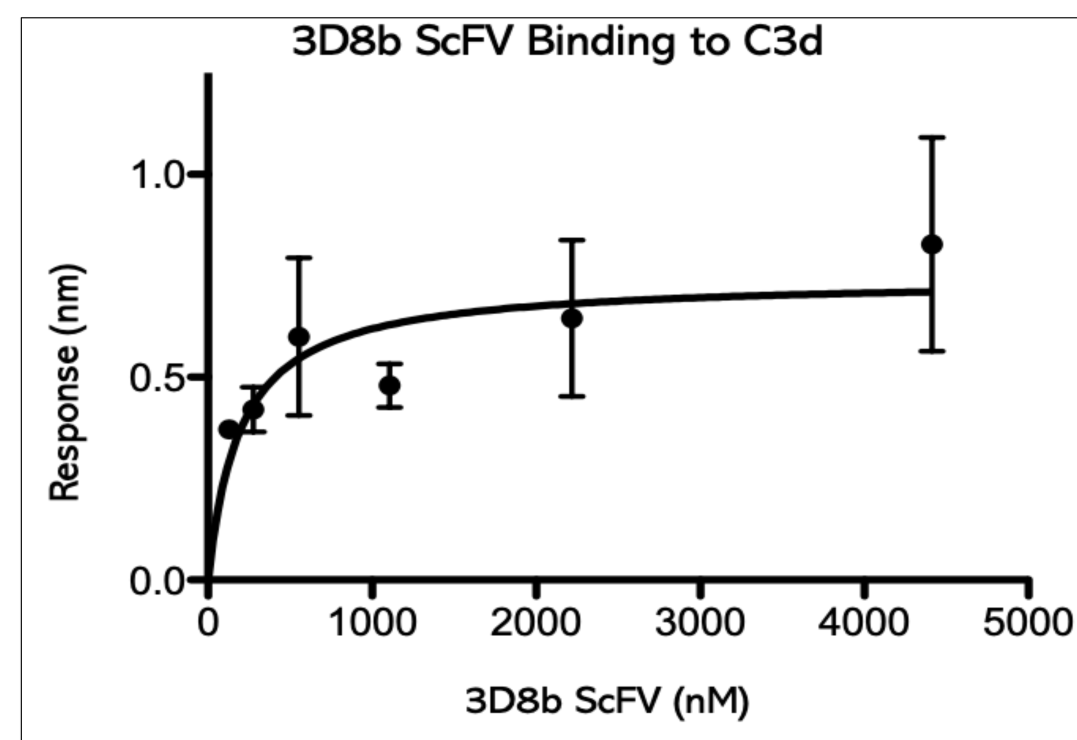

Figure 3: Binding affinity between GST-C3d and 3D28b. K_D value was calculated to be 199.1 ± 158.9 nM.

Results: Molecular Docking, Simulated Binding

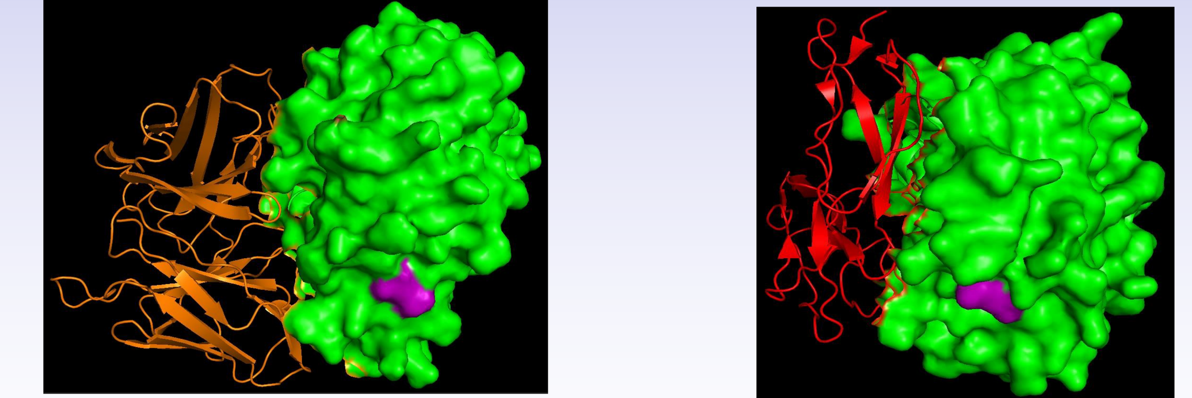

Figure 6: (left) Rigid-rigid- dock of 3D29 SWISS-MODEL (orange) with the known crystallized structure of *Homo sapiens* C3D (green). This dock had a HADDOCK score of 34.0 +/- 15.7. **(right)** Complex between C3D (green) bound to its natural complement receptor CR2 (red). The first residue of C3D has been colored purple for better ease of comparison.

Methods

Mammalian Cell Transfection:
Transfection media was prepared by placing DMEM (no additives) in two tubes equal to 1/20 culture volume in each tube. To one tube 1μg of 3D29 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 3D29 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 s200 increase size exclusion column.

Expression and Purification of Human C3d:
C3d was expressed using the pGexsystem, thus tagging the C3d with Glutathione S-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 (ForteBio) using the Simple Kinetics protocol. GST-C3d was bound to 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 (3D29 and 3D8b) and C3d
-Prepare crystal trays to begin process of crystallization and diffraction visualization

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UCCS University of Colorado Colorado Springs