Towards Understanding the EBV gp350 – Complement Receptor 2 Interaction

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Introduction

- Epstein-Barr Virus (EBV) is one of the most common viruses in humans. EBV is also associated with many cancer types (2), conditions of human immunodeficiency virus (HIV) (3), and autoimmune diseases (4). Over 200,000 new cases of cancer related to EBV are reported each year (10).
- Infection by EBV is mediated through the binding of glycoprotein spike gp350 with a specific immune cell receptor responsible for normal, robust immune responses and building memory to pathogens: Complement Receptor 2 (CR2) (5-9). This makes inhibiting the molecule difficult because inhibition of the entire CR2 molecule would inhibit immune response.
- We are in the process of determining a 3-D model of gp350 bound with CR2. This may provide insight into how the proteins bind and areas that can be inhibited without hindering immune response.
- The short consensus repeats (SCR) of CR2 believed to bind with gp350 are SCR 1-2 (11). CR2 SCR 1-2, is synthesized instead of complete CR2.

Objective: The research will examine affinity and binding of the gp350-CR2 complex by creating a 3-D complex of the proteins via crystallography.

Methods

Expression of CR2

- The CR2 plasmid vector necessary for transfection had been synthesized beforehand. The gene has a His-tag gene for affinity column purification.
- Split 293T cells into 8225 cm^2 culture flasks. Once the cells reached 90% confluency the cells were transfected with CR2 (SCR1-2) DNA and after 24 hours, media was exchanged with freestyle media.
- The His-CR2 SCR 1-2 was collected, purified via a Ni^{2+} affinity column, concentrated, and collected using FPLC with a Superdex 200 column.
- Expressed MBP-CR2 SCR 1-2 protein via bacterial cell expression followed by MBPTrap-HP column purification and FPLC collection.

gp350 Plasmid Synthesis and Expression

- Performed BP and LR reactions on His-gp350 plasmid to transfer the gene to the pVRC-8400 destination vector.
- We have performed gene sequencing and a restriction digest on the vectors to confirm the His-gp350 has been successfully cloned.
- The His-gp350 was expressed, purified and collected using the same methods as the His-CR2 protein.

Works Cited

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List of other references provided on handout sheet.

Results

FPLC Results:

- The FPLC graphs can also determine the relative size of the proteins based on the location of their peaks on the graph. The relative kDa mass of His-gp350 and His-CR2 SCR1-2 peaks were found in their FPLC graphs.
- The MBP-CR2 SCR 1-2 collected appeared to aggregate resulting in large peaks around 669 kDa rather than the expected 61 kDa.

Protein Gel Results:

- His-CR2 SCR 1-2 have a molecular weight of 17 kDa but appears to be ~34 kDa on the protein gel suggesting dimerization.
- MBP-CR2 SCR 1-2 has a molecular weight of ~61 kDa and these bands appear on the protein gel suggesting successful synthesis of MBP-CR2.
- His-gp350 has a molecular weight of ~104 kDa due to glycosylation which was indicated on the protein gel.

Bio-layer interferometry (BLItz) Analysis:

- BLItz analysis was used to study the binding of gp350 to CR2 SCR 1-2 by determining the K_D of the protein-protein binding interaction.
- The K_D values were relatively large indicating weak binding.

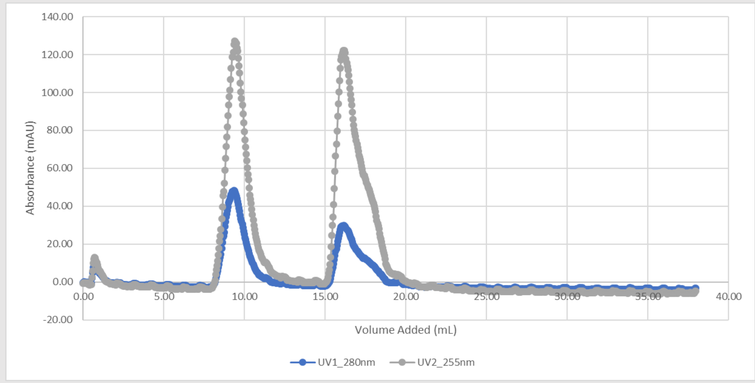

Figure 1: Resolved the purified MBP-CR2 SCR 1-2 by gel filtration chromatography on a Superdex 200 Increase column via injection into the Äkta explorer FPLC.

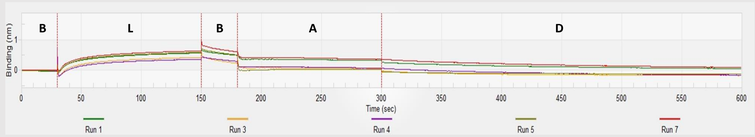

Figure 2: A BLItz analysis graph showing the baseline measurements of the biosensor (B), loading of His-gp350 (L), association of MBP-CR2 with gp350 loaded onto the biosensor (A), and the dissociation of MBP-CR2 from gp350 (D). Note: runs 2 and 6 were excluded due to inaccurate baseline measurements. His-gp350 was loaded to an anti-His biosensor.

Run	His-gp350 (nM)	MBP-CR2 (nM)	K_D (µM)
1	2000	2000	$2.166*10^4$
3	2000	1000	$3.038*10^4$
4	2000	500	$4.695*10^2$
5	2000	250	$<1*10^{-6}$
7	2000	125	$1.019*10^3$

Figure 3: The K_D values measured from the BLItz analysis graph in figure 2. The gp350 concentration was kept constant while the MBP-CR2 concentration was changed to see if maximum binding truly occurs in a 1:1 concentration.

Future Research

- The binding of gp350 to MBP-CR2 was weaker compared to the literature values (~0.077 µM) which was likely due to interference from the 125 mM NaCl concentration (11). Future BLItz analysis studies will use a 50 mM NaCl concentration to lower interference and provide a more accurate comparison to the literature values.
- A protocol for crystallography has been found and will be implemented for crystallography to make a 3D crystal structure of the gp350/CR2 SCR1-2 complex.
- After Identifying the 3D crystal structure between gp350 and CR2 SCR 1-2, inhibitors will be investigated which can inhibit the gp350 and CR2 SCR 1-2 interaction without inhibiting memory to pathogens and immune response.

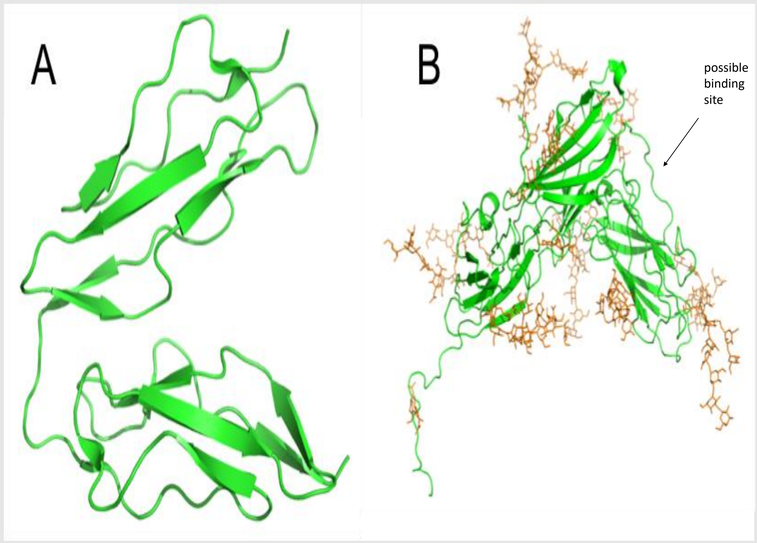

Figure 4: **A.** CR2 SCR1-2 **B**. EBV gp350.

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