University of Glasgow | College of Medical, Veterinary & Life Sciences

Head of College Scholars List Scheme

Summer Studentship

Report Form

This report should be completed by the student with his/her project supervisor. It should summarise the work undertaken during the project and, once completed, should be sent by email to: jill.morrison@glasgow.ac.uk within four weeks of the end of the studentship.

1. Student

   Surname: Bolton Forename: Victoria Lena

   E-mail address: 2091488B@student.gla.ac.uk

2. Supervisor:

   Surname: Patel Forename: Arvind

   E-mail address: Arvind.Patel@glasgow.ac.uk

3. Research Project Report

   3.1 Project Title (maximum 20 words):

   Identification of epitopes of a hepatits C virus neutralizing antibody (CBH-7) with a view to vaccine development.

   3.2 Project Lay Summary (copied from application):

   Hepatitis C virus (HCV) causes liver cirrhosis and cancer. Expensive treatments are available but there is still no vaccine. A few infected individuals do clear infection without treatment, this is associated with the early appearance of broadly neutralising antibodies (bnAb) targeting the viral protein E2. One vaccine approach is to harness natural immunity against

regions on E2 that elicit bnAb. The human bnAb CBH-7 binds to HCV E2 protein and neutralizes HCV infection, however the binding site is not known. This project will use a library of peptides spanning E2 to identify important regions for CBH-7 binding.

3.3 Start Date: 15/6/2015 Finish Date:7/8/2015

3.4 Original project aims and objectives (100 words max):

The binding site of bnAB CBH-7 binding of E2 is still unknown. The aim of this project therefore was to identify the key epitope residues that define the binding site of bnAB CBH-7 on E2.

Key questions:

- What are key epitope residues in bnAB CBH-7 binding of E2?

3.5 Methodology**:** Summarise and include reference to training received in research methods etc. (250 words max):

Preparation of E1E2 plasmid DNA: Competent *E.coli* were incubated with plasmid DNA on ice for 30mins, heat-shocked at 42oC for 1 min and rested on ice before plating onto LB-Agar plates containing Ampicillin. Plates were incubated overnight at 37C. LB-broth was inoculated with a single colony and grown at 37C on a shaker overnight. Plasmid DNA was extracted using the Qiagen spin miniprep kit as per the manufacturers' protocol. DNA concentration was quantified by measuring absorbance at 260nm.

Production of E1E2 lysate: HEK 293T cells were transfected with E1E2 plasmid DNA using calcium phosphate transfection and incubated overnight at 37C. The transfection mix was replaced with fresh media and incubated for 24h. Cells were lysed for 20min, lysate was cleared by centrifugation (13000g, 10mins, 4C).

GNA capture ELISA assay: Immulon II enzyme immunoassay plates were coated with Galanthus nivalis (GNA) lectin. Plates were blocked with 1% skimmed milk powder in PBS containing 0.1% Tween (PBS-T). E1E2 lysate was incubated at RT for 2h. Plates were washed in PBS-T (x3), incubated with an E2 antibody for 1h, washed and incubated in secondary HRP-conjugated antibody for 1h. The plate was washed in PBS-T (x6) then TMB substrate was added, the reaction was developed for 20min then stopped with 0.5M H_2SO_4. Absorbance was measured on a platereader at 450nm.

Peptide library screen: The standard GNA ELISA assay was adapted as follows. H77 genotype 1a E1E2 lysate was used. Human bnAB CBH-7 at 0.1 ug/ml in the presence of 10 ug/ml peptide in 100ul of PBST was bound for 1h at RT. Bound antibody was detected with human HRP-antibody Promega W 4031.

3.6 Results: Summarise key findings (300 words max). Please include any relevant tables or images as an appendix to this report:

Peptide library screen:

A library of peptides spanning HCV E2 were analysed in a competition E1E2 ELISA assay, with the aim of identifying the E2 region containing the CBH-7 epitope. Genotype 1 H77 E1E2 lysate bound plates were incubated with Hmab CBH-7 in the presence of E2 peptide. Competition for binding between Hmab CBH-7 and a peptide would be reflected in a lower absorbance value. No inhibition was observed with the samples containing CBH-7 and peptide. The positive control , Mab V3.2 however, in the presence of peptide 25, showed significant inhibition confirming the assay was working (see Figure 1). These results confirm that CBH-7 binds to a conformational epitope as opposed to a linear epitope.

GNA capture ELISA essay:

An alternative strategy to identify residues that form part of the conformational epitope of CBH-7 used a panel of genetically variable E1E2 proteins, cloned from clinical cohorts. The expression levels of E2 were normalised using the linear anti-E2 Mab AP33 to a signal of 2 OD, to serve as a reference value. In parallel, Mab CBH-7 binding was measured. The panel of E1E2 proteins were ranked according to the average relative binding affinity to CBH-7 then grouped into 3 groups: Increased binding, normal binding, and decreased binding according to their relative binding affinity to CBH-7 compared to their binding affinity to Ap33 (Figure 2).

Alignment of amino acid sequences of each E1E2 protein according to their ranking revealed patterns of amino acid grouping that correlate with the increase or decrease in binding affinity of CBH-7 (Figure 3). Figure 3 shows E2 positions of special interest with known surface amino acid residues e.g. 434, 442, 533, 560 highlighted in red. E1E2 protein variants with decreased CBH-7 binding affinity demonstrate acidic amino acid residues at position 434 as opposed to non-acidic residues carried by E1E2 protein variants with increased binding. At position 442 a change from phenylalanine to leucine was recorded for E1E2 variants 12.20 and UKN 220, that both showed an increase in CBH-7 binding affinity. Moreover, the presence of a tyrosine at position 560 correlated mostly with an increased binding affinity of E1E2 variants carrying this amino acid.

The position of these residues of interest within the E2 protein, were investigated with the molecular modelling program Chimera 1.9 and are displayed in Figure 4. Residue 434 and 560 are closely associated with each other on the surface of the protein. Moreover residue 434 forms a close interface with epitope II.

Analysis of predicted CBH-7 glycosylation sites showed that N- glycosylation was reduced at position 448 and 540 for lysates that were poorly bound by CBH-7 (Table 1).

3.7 Discussion (500 words max):

The aim of this project was to investigate and determine possible CBH-7 binding sites. Earlier studies (Keck et al. 2004) have suggested that CBH-7 binds to a conformational epitope in Domain C, that differs spatially from other antibody binding domains A and B as determined by competition studies. The peptide screen conducted in this project investigated competition for CBH-7 between H77 E1E2 protein and linear peptides spanning the E1E2 protein and found no inhibition providing further evidence that CBH-7 binds to a conformational epitope.

GNA capture Elisa assay and downstream sequence analysis have identified positions 434 , 442, 533 and 560 as putative CBH-7 interacting residues. Residue 533 falls within a region of E2 involved in binding the receptor CD81. In addition, this residue is in close proximity to residues of Domain A (Keck et al. 2012), which has been suggested to be in proximity to Domain C in previous competition studies (Keck et al. 2004). Furthermore, residues 434 and 560 are in close proximity with epitope 2/ Domain B which includes a CD81 interaction site. The same study (Keck et al. 2004) have suggested that CBH-7 inhibited E2-CD81 interactions suggesting that residues 533, 434 and 560 could be of further interest. Further experiments including site-directed mutagenesis, mutating E1E2 variants of increased binding and decreased binding by CBH-7 (Figure 2) at residues 434 , 442, 533 and 560 to an alanine could be conducted to investigate the effects on CBH-7 binding.In addition, other residues of interest 416, 463, 466, 475, 481 could also be investigated by site- directed mutagenesis to investigate their effect on CBH-7 binding. These residues are not included in the E2 core protein structure, therefore it is not known for certain if these residues are surface-accessible for antibody interaction . However these residues may be located on the surface of the E2 protein and therefore may be putative CBH-7 interaction sites.

Analysis of the N-glycosylation pattern of E1E2 proteins, ordered according to ranking of CBH-7 binding from highest to lowest revealed E2 residues 448 and 540 as of possible interest. Both residues were more likely to be glycosylated in E1E2 variants that had reduced CBH-7 binding affinity (Table1). Interestingly these sequences were also less likely to have glycosylation sites at two further residues within E1 (196 and 234). The effect of N-glycosylation on CBH-7 binding could be investigated by knocking out glycosylation at residues 448 and 540 from E1E2 variants e.g. 1202 and UKN 220 that were bound well by CBH-7 by mutation of the asparagine residues. Finally, it would be interesting to create chimeras of E1E2 variants that combine different E1 and E2 glycosylation sites of interest e.g. 193, 234 and 448 and 540 to investigate this further.

4. Reflection by the student on the experience and value of the studentship (300 words max):

When I started the project I had little experience in working in a laboratory and related skills and I was excited, but also nervous about my placement.

However through the work on my project , the support which I received from my friendly and helpful colleagues and the direction of the supervising postdoc Vanessa Cowton in the group, I soon felt integrated into the team.

During my placement I learned a number of techniques and was able to develop skills that will be useful in my further studies and career, e.g ELISA assays, transfections and basic cell culture techniques. The tasks I were given in my project were both interesting and challenging and as my placement progressed my confidence in carrying them out increased. Even when I made mistakes I was encouraged and assisted in solving the problems encountered in these experiments , which gave me experience in both troubleshooting and avoiding these mistakes. I could closely identify with the aim of the project and found conducting my experiments led me both to learn a great deal and feel that I was making a

useful contribution. For example it was a valuable insight to discover that not all results are consistent and that repeating an experiment many times to secure reliable results is a key facet of lab work.

It was great to not only work on my own small project and learn from my mistakes, but also to get an insider's view of both the general research lab environment and the projects of my colleagues and the other research groups when they presented their work during the weekly seminars which I was able to attend at the beginning of my summer placement. This experience has encouraged me to continue my studies in the field of infection biology and aim for PhD after my undergraduate degree.

This summer internship has showed me how valuable lab skills are and I will apply for a placement year in a lab environment as part of my degree.

In conclusion, this summer's project placement has been both challenging and inspiring. I feel that I gained many useful skills that will help me in my continuing studies and career and I am looking forward to the opportunity of a further summer placement.

5.Dissemination: (note any presentations/publications submitted/planned from the work)

N/A

6. Signatures:

Supervisor ARVIND H PATEL Date 14TH August 2015

Student VICTORIA LENA BOLTON 14TH August 2015

Appendix:

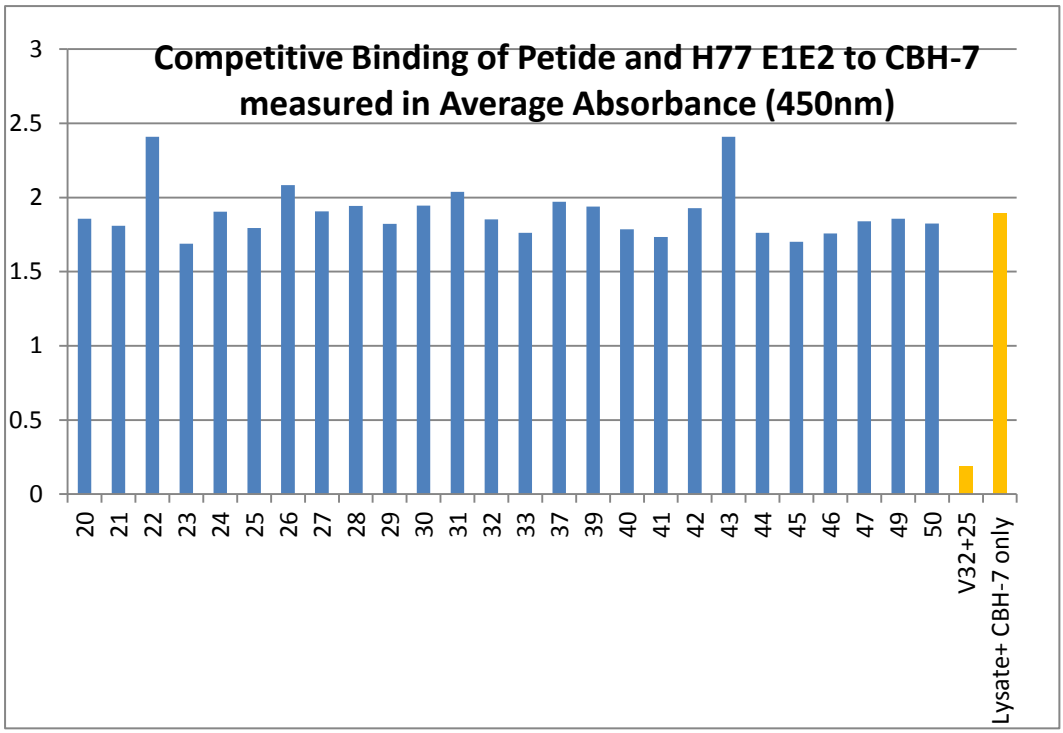

Figure 1: *Competitive ELISA assay. Competitive binding of peptides spanning E2 and H77 E1E2 to CBH7 measured in Average Absorbance (450nm) in OD in blue. Controls in yellow: A. Competitive binding of peptide 25 plus H77 E1E2 to antibody V32. B. H77 E1E2 plus CBH-7 antibody only.*

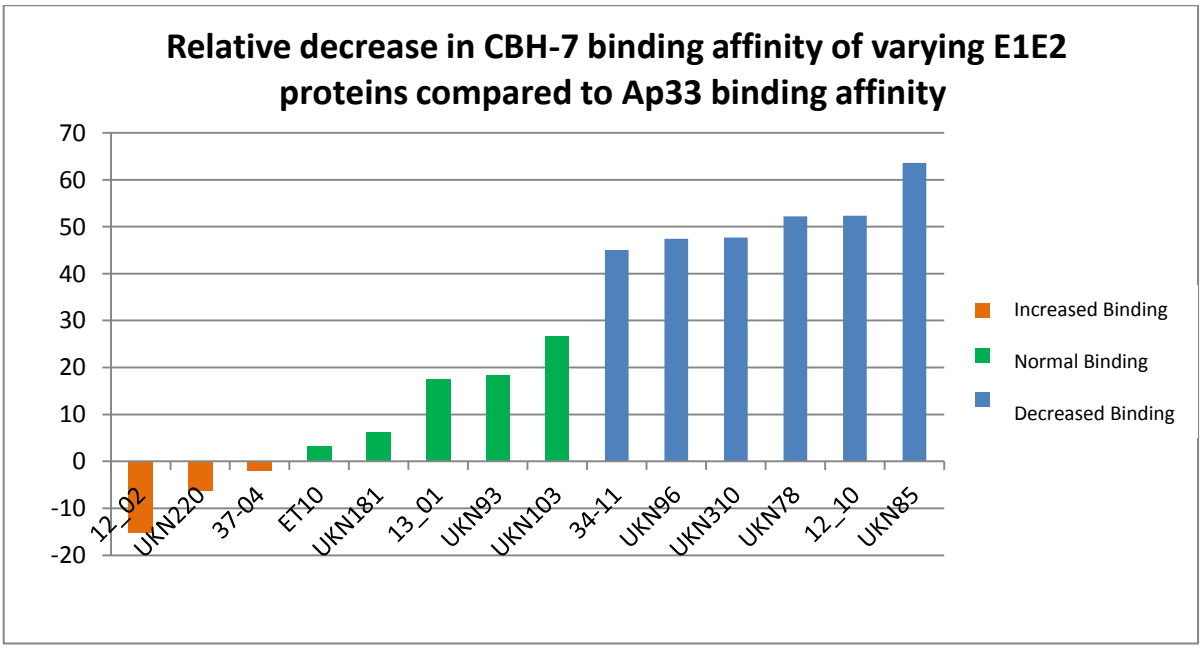

Figure 2 : *Relative decrease in relative binding of CBH-7 to E1E2 variants as compared to binding of Ap33 to E1E2 variants Orange E1E2 variants are better bound by CBH-7. Blue E1E2 variants are bound significantly worse by CBH-7.*

	ND	no	+	no		ND	ND	ND	ND	+	No	No	No	+	No	Adjacent to surface region
Position	416	431	434	438	442	463	466	475	481	533	537	557	558	560	610	626
E1E2 protein lysates																
12_02	S	E	T	V	L	T	A	T	D	E	F	A	T	Y	D	I
UKN220	S	D	T	I	L	T	D	T	D	E	F	A	T	Y	D	I
37-04	S	D	H	I	F	A	A	T	D	E	F	A	T	Y	D	I
ET10	T	D	Q	I	F	T	A	T	D	D	F	S	T	F	D	I
UKN181	T	D	H	I	F	T	D	V	D	E	F	S	T	F	H	V
13_01	T	A	D	V	F	A	A	A	G	D	F	S	S	F	H	L
UKN93	T	A	D	V	F	A	D	A	E	D	L	S	S	F	H	M
UKN103	T	A	D	V	F	A	D	A	E	D	F	S	S	F	H	L
34-11	T	A	E	V	F	A	D	A	E	D	F	S	S	F	S	L
UKN96	T	A	D	V	F	A	D	A	E	D	L	S	S	F	H	M
UKN310	T	A	D	V	F	A	D	A	E	D	F	S	S	F	H	L
UKN78	T	A	D	V	F	A	D	A	E	V	L	S	S	F	H	L
12_10	T	A	E	V	F	A	D	A	D	E	F	A	T	Y	N	I
UKN85	T	A	D	V	F	A	D	A	E	D	L	S	S	F	H	L

no	**no surface region**	
+	surface region of special importance	
	surface region	
ND	not displayed in E2 structure	
D/E	Acidic residues	
H	Basic residues	

Figure 3: *Alignment of Amino acids of genetic variants of E1E2 at specific positions of E2.*

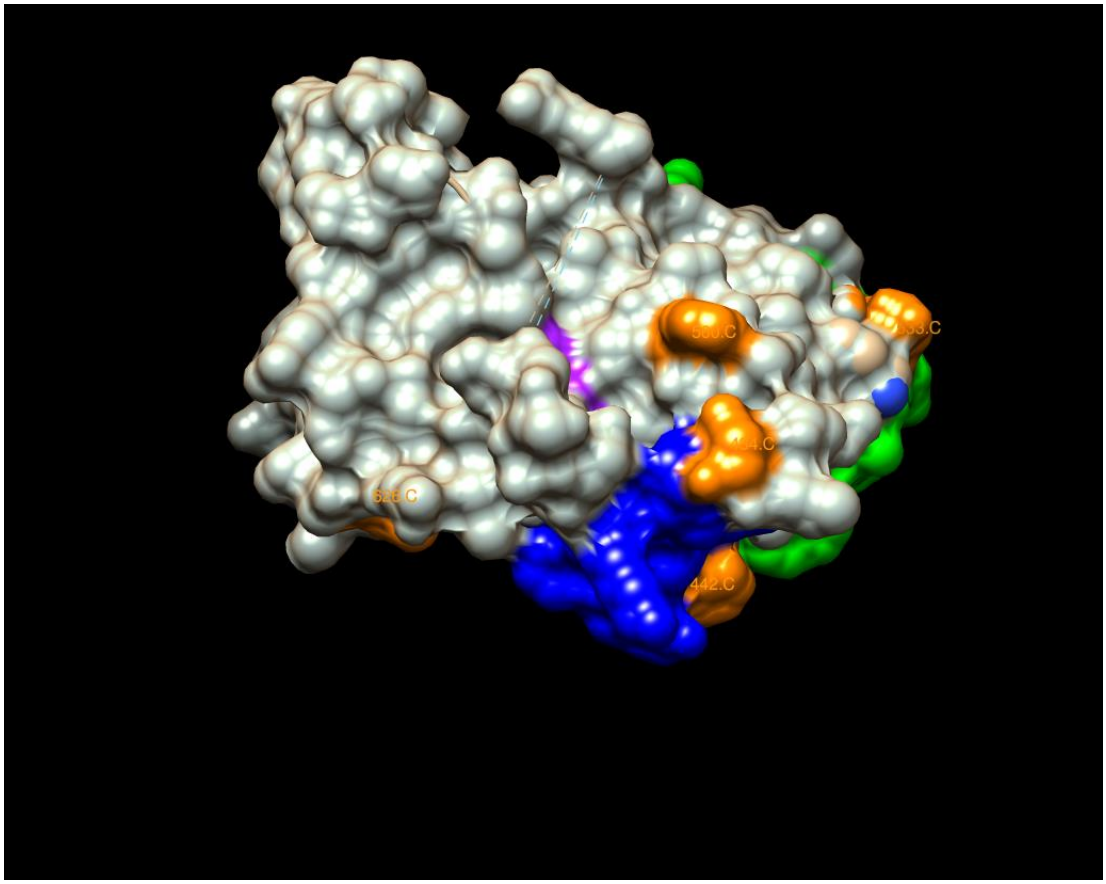

Figure 4 *Image of E2 core structure from Chimera. Blue region is epitope II = domain B, Orange is putative CBH-7 interacting residues.*

Table 1: *Predicted CBH-7 glycosylation sites. N for N- glycosylation. E1E2 variants that are increasingly bound by CBH-7 in orange. Intermediate/normal E1E2 variants in green and E1E2 variants that are not well bound by CBH-7 in blue.*

	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	196	209	**234**	250	305	367	417	423	430	**448**	476	532	**540**	556	576	623	645
12.02	Y	Y	Y	N	Y	N	Y	N	Y	Y	Y	Y	N	Y	Y	Y	Y
220	Y	Y	Y	N	Y	N	Y	N	Y	N	N	Y	N	Y	Y	Y	N
37-4	Y	Y	Y	N	Y	N	Y	N	Y	N	Y	Y	N	Y	Y	Y	Y
ET10	Y	Y	Y	N	Y	N	Y	N	Y	N	N	Y	N	Y	Y	Y	Y
181	Y	Y	Y	N	Y	Y	Y	N	Y	N	Y	Y	N	Y	Y	Y	Y
13_01	Y	Y	Y	N	Y	N	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	N
UKN93	N	Y	N	N	Y	N	Y	N	Y	N	Y	Y	Y	Y	Y	Y	N
UKN103	N	Y	N	N	Y	N	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y
34-11	Y	Y	Y	N	Y	N	Y	N	Y	Y	Y	Y	N	Y	Y	Y	Y
UKN96	N	Y	N	N	Y	N	Y	N	Y	N	Y	Y	Y	Y	Y	Y	N
UKN310	N	Y	N	N	Y	N	Y	N	Y	Y	Y	Y	N	N	Y	Y	N
UKN78	N	Y	N	N	Y	N	Y	N	Y	Y	Y	N	Y	Y	Y	Y	Y
12.10	Y	Y	Y	N	Y	N	Y	N	Y	Y	Y	Y	N	Y	Y	Y	Y
UKN85	N	Y	N	N	Y	N	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	N