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Human Monoclonal Antibodies for the Treatment and Prevention of Crimean Congo Hemorrhagic Fever

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INTRODUCTION

- Crimean-Congo hemorrhagic fever (CCHF) is the most widely spread tick-born viral hemorrhagic disease, with high fatality rates of up to 50% in hospitalized patients.
- Currently there are no approved vaccine or therapeutics available for this disease.
- The CCHFV glycoprotein is made up of two major structural glycoproteins, G_C and G_N. An additional secreted protein, GP38, has an unknown role in viral replication and pathogenesis (**Figure 1**).
- 13G8, a non-neutralizing mouse mAb, targets GP38 and protect up to 60-90% in a mouse model.
- Neutralizing mouse mAbs to G_C have not shown consistent protection in mouse.
- We isolated and characterized mAbs from human survivors of CCHF.

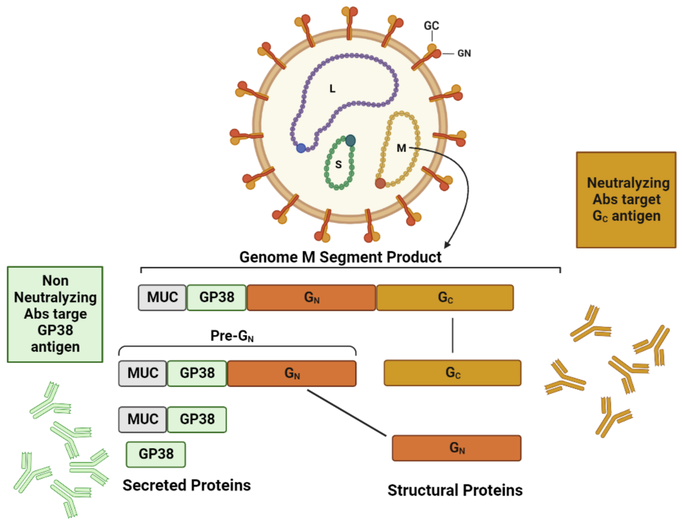
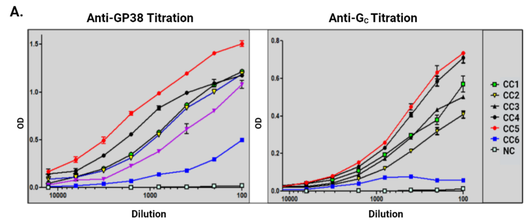


Figure 1. A schematic depiction of Each CCHF virus. Spikes of glycoproteins GN and GC are embedded in its lipid envelope. The virion contains three single-stranded RNA genome segments (**S**mall, **M**edium, and **L**arge). The M segment encodes the glycoprotein precursors. Antibodies against Gp38 do not neutralize but may protect.

Methods and Results

Method. The antibody response to GC and gp38 antigens were measured in CCHFV patients from Turkey ~ 6 months after infection, and one-high titer sample was selected for mAb isolation. For this purpose, a combined proteomics and genomics approach was performed; plasma Abs were isolated using sequential affinity chromatographies, and the peptides subjected to Mass spectrometry, and then matched to single cell VDJ sequence libraries constructed from PBMCs (**Figure 2**). The isolated mAbs were tested for binding affinity and neutralization, and the neutralzing antibodies were tested for eppitope mapping. Finally one candidate used for *in vivo* evaluation. For this purpose, the CCHFV Turkey-2004 strain was injected subcutaneously into IFNAR-/- mice (target dose: 100 TCID50) and treatment was given intraperitoneally with either 13G8 or CC5-17 (n = 6). The mice were monitored for 21 days post-challenge.

Results. 11 anti-GP38 and 8 anti-Gc antibodies were isolated. All anti-Gc (and none of the GP38) mAbs exhibited neutralization. The anti-GP38 antibodies were segregated into 5 epitope groups based on competition assays, (including 2 not described before). CC5-17, the human mAb sharing the same epitope as 13G8, but with much higher affinity, showed 50% protection. two separate experiments in Ifnar1^{-} mice (when given 30 minutes prior to challenge, or 1 and 4 days after challenge).



B.

Saturating mAb		Competing mAb: 13G8	CC5-17	CC5-6	CC5-12	CC5-16	8F10	CC5-25	7F5	CC5-14	CC5-20
			Site I			Site V	Site II	Site III		Site IV	
Site I	13G8	-1	-22	-20	-7	83	85		307	149	320
	CC5-17	6	-8	-9	6	249	100	171	66	165	144
	CC5-6	11	8	10	33	176	88	110	88	126	129
	CC5-12	-4	-31	-33	-4	26	13	132	153	146	129
Site V	CC5-16	2	93	105	32	-17	-4	151	130	138	138
Site II	8F10	61	346	106	120	73	-6	-3	203	120	428
Site III	CC5-25	74	149	56	175	228	-4	16	51	147	130
	7F5	68	153	80	259	151	145	33	30	88	96
Site IV	CC5-14	66	127	111	171	229	117	112	17	-11	-1
	CC5-20	68	122	81	158	202	32	109	62	-16	-11

Figure 3.
A) Titration of anti-GP38 and Gc antibodies in 6 CCHF survivors in Trabzon (Turkey). The CC5 patient shows the highest titer of both antibodies.
B)7 anti-GP38 CC5 antibodies were then tested against known Group 1, Group 2 and Group 3 antibodies. <33% binding indicates competition, 34-66% indicate partial competition, >67% indicate no competition

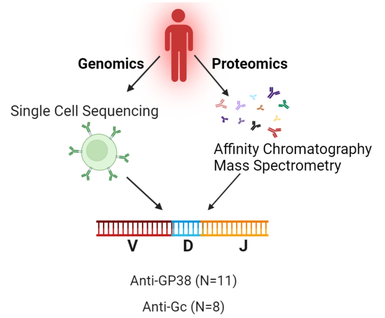


Figure 2. Schematic depiction of the applied proteogenomics approach used for antibody isolation.

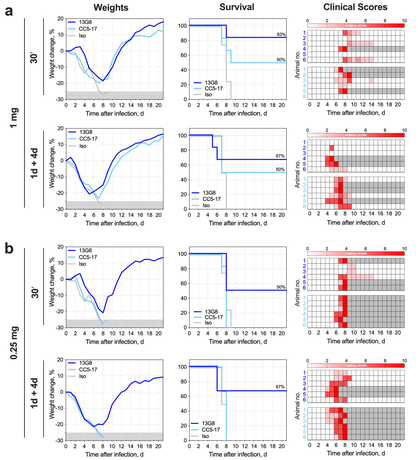


Figure4.
The mean weight change, survival rate, and clinical scores in mice were examined followed. **a**, 1 mg mAb treatment at 30 min or +1/+4 days after challenge or **b**, 0.25 m mAb dose treatment at 30 min or +1/+4 days after challenge.

Discussion

Crimean Congo hemorrhagic fever virus (CCHFV) causes a viral infection that is frequently life-threatening. CCHFV is a tick-borne disease that is endemic to a wide geographical area along with the possibility of expansion. At present, CCHFV does not have a proven antiviral treatment, so supportive care is the primary mode of disease management.

The lack of antivirals has led to monoclonal antibodies being considered a viable treatment modality. Although neutralizing antibodies have beneficial therapeutic properties against some viruses, there is growing evidence supporting the therapeutic role of non-neutralizing antibodies towards preventing severe diseases. Studies conducted on anti-GP38 mAb have shown that this antigen is an effective target for this purpose.

Here, we isolated and characterized several anti-GP38 and anti-GC antibodies from human reservoirs and identified CC5-17 as a non-neutralizing GP38 antibody that binds the same epitope (Site I) as 13G8.

Our data demonstrates that non-neutralizing GP38 antibodies provide protection before or after exposure to CCHFV *in vivo*. In spite of CC5-17's high affinity for the GP38, it confers lower therapeutic efficacy than 13G8, indicating that affinity for non-neutralizing antibodies to GP38 is not the sole factor determining protective activity. Furthermore, the findings suggest that there are likely to be other factors at play.

An antibody derived from humans that provides partial protection in animals illustrates the importance of targeting the GP38 antigen specifically at site I for therapeutic antibodies and rational vaccine design. We plan to isolate and characterize a larger number of anti-GP38 antibodies in light of the promising results of this study, as well as test their efficacy in protecting against CCHFV in the future.