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Isolation of anti-CCHF B cell lymphocytes from a convalescent South African survivor by single-cell analysis

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Crimean-Congo haemorrhagic fever (CCHF) is a life-threatening anthropo-zoonosis that is characterized by high fatality rates 5-40%. Currently there are no approved vaccines or treatment available. Recently, antibody-based therapies have proved promising approaches to mitigating severe disease caused by other haemorrhagic viruses . CCHFV is made up of two structural glycoproteins, Gc and Gn, the sole targets of neutralizing antibodies and the nucleocapsid protein (NP), which is antigenically well-conserved among different strains. This study seeks to utilize Fluorescence Activated Cell Sorting (FACS) analysis to isolate Gc, Gn and NP targeting resting memory B cells from PBMCs of a convalescent donor.

In this study, Gc and NP were cloned into pcDNA3.1+ and the resulting recombinant plasmids were used to transfect 293-F mammalian cells. Both Gc and NP proteins were purified by affinity chromatography and gel filtration, and the donor serum IgG reactivity to the antigens was measured by ELISA. Gc and NP were further biotinylated in preparation for B cell sorting and verified by ELISA on an avidin coated plate. The donor PBMCs were stained with anti-human APC-Cy7 labeled CD3, CD14, CD16, PE-Cy7 CD19, and IgD-FITC, as well as the Gc/NP-PE/AF647 antigen baits, and the LIVE/DEAD stain prior to loading onto a BDFACS Melody. The total Gc/NP- specific B cells CD19+ were sorted into 96-well plates for cloning and expression of anti-CCHF monoclonal antibodies (mAbs).

CCHFV Gc and NP were successfully cloned, expressed and purified as confirmed by SDS-PAGE and Western blotting. ELISA confirmed the presence of binding anti-CCHF IgG antibodies in donor sera. The biotinylation of both Gc and NP was successful, and these antigens were used to isolate B cell lymphocytes from a South African CCHF survivor to isolate potent and protective mAbs by single-cell analysis.

We have successfully expressed CCHFV Gc and NP which can be used for various applications including antigen-targeted B cell isolation. Furthermore, these antigens constitute valuable reagents for the development of diagnostic assays like ELISA and lateral flow assays which could be useful in low-income sub-Saharan African countries.

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